ÉTUDE DU PATRON D'INVASION DES VERS DE TERRE EXOTIQUES DANS LE PARC NATIONAL DU MONT-TREMBLANT ET DE LEURS IMPACTS SUR LE MILIEU FORESTIER

par

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Mémoire présenté au Département de biologie en vue
de l'obtention du grade de maître ès sciences (M.Sc.)

FACULTÉ DES SCIENCES
UNIVERSITÉ DE SHERBROOKE

Sherbrooke, Québec, Canada, 16 novembre 2015

Le 16 novembre 2015

le jury a accepté le mémoire de Madame Martine Fugère
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Au Québec, les premiers Lumbricidae ont été introduits par les colons européens aux alentours du 16^{e} siècle. Depuis cette première vague d'introduction, ils se propagent sur le territoire à l'aide de l'activité humaine. Cependant, nous en savons peu sur l'effet des diverses activités humaines sur l'établissement des communautés de Lumbricidae en milieu forestier protégé. De plus, leurs effets sur les cycles biogéochimiques et les plantes de sous-bois restent peu connus. Le premier objectif de notre étude était de comparer l'effet de la circulation routière et du transport d'appâts par les pêcheurs sur l'abondance et la structure des communautés de Lumbricidae en forêt. Notre deuxième objectif était de vérifier si les vers de terres exotiques pouvaient influencer la production d'oxyde nitreux, un gaz à effet de serre important. Enfin, le dernier objectif était de mesurer l'impact des vers de terre sur la richesse et la composition du sous-bois forestier.

Nous avons réalisé un échantillonnage autours de 61 lacs du parc national du Mont-Tremblant, 46°26′00″N 74°21′00″W, avec différents niveaux d'activité humaine. Nos résultats montrent que la pêche, la proximité des routes et l'acidité du sol ont tous les trois un effet positif sur l'abondance des vers de terre. Cependant, la pêche augmente davantage la densité et la richesse d'espèces de vers. De plus, seule la pêche semble permettre l'introduction des vers anéciques *Lumbricus terrestris*. Afin d'estimer l'impact des vers de terre sur la biodiversité végétale et les propriétés du sol nous avons échantillonné 47 points autour de deux lacs envahis pour observer un gradient d'invasion. Nos tests ont montré que l'abondance de *Lumbricus terrestris* était corrélée positivement avec les taux de nitrification et de dénitrification dans le sol, deux processus pouvant émettre de l'oxyde nitreux vers l'atmosphère. Une augmentation de l'abondance des *Lumbricus terrestris* était également corrélée avec une diminution de la richesse d'espèces herbacées. Nous avons observé que les abondances combinées des vers anéciques et endogéiques influençaient la composition de la végétation de sous-bois. Leur abondance était corrélée négativement avec le recouvrement de

Maïanthemum canadensis, *Trientalis borealis* et *Dryopteris intermedia* tout en étant corrélée positivement avec celui de *Carex sp*. Les résultats de notre étude pourront être utilisés par nos partenaires de la SÉPAQ afin d'assurer une meilleure gestion de la problématique des vers de terre exotiques. Nos résultats militent en faveur d'un moratoire sur la création de nouvelles routes et sur l'utilisation de vers vivants en tant qu'appâts dans les parcs nationaux. De manière plus générale, cette étude amène des pistes de solutions quant à la gestion de la propagation des vers de terre exotiques dans des nouveaux milieux, par exemple en donnant aux autorités nécessaires le mandat de contrôler l'accès des pêcheurs dans les secteurs non envahis.

Mots-clés : vers de terre, espèce exotique, pêche, gaz à effet de serre, aire protégée, activité humaine, herbacées

REMERCIEMENTS

Je remercie mon directeur Robert Bradley pour l'opportunité de ce projet de recherche vraiment enrichissant qui m'a permis de me dépasser. Je voudrais remercier chaleureusement mon co-directeur Mark Vellend, qui malgré une année sabbatique en Australie a trouvé le moyen d'être toujours présent pour m'assister dans mon projet. Je remercie également les membres de mon comité conseil, Bill Shipley et Marc Bélisle pour leurs idées novatrices et leur appui. Je remercie Bill Parson pour ses judicieux conseils et sa précieuse aide en laboratoire. Je remercie Benoît Lapointe, l'homme à tout faire du laboratoire (même les miracles!) pour son aide et ses enseignements (tel que la conduite de vtt). Je remercie Mathieu Dufresne pour ses enseignements précieux quant au travail de terrain. Je remercie aussi Cédric Frénette Dussault pour ses conseils en analyses statistiques.

Je remercie de tout cœur Patrick Graillon grâce à qui ce projet en collaboration avec la SEPAQ a pu être possible. Je remercie également Hugues Tennier et Émilie Dorion pour leur aide lors de la planification du terrain. Je remercie aussi tous les gens de la SEPAQ avec qui j'ai eu la chance d'interagir lors de mon terrain et qui mettaient un peu de soleil dans mes journées pluvieuses!

Je remercie tous les stagiaires de terrain, Gerard, Laurence, Céline, Geneviève et Alexandra ainsi que Mathieu Gauthier et Charline Bringuier pour leur aide précieuse sur le terrain. Je remercie également Charline pour ses sages conseils et son appui lors des moments plus difficiles. Enfin, je remercie les membres de ma famille et mon amoureux Olivier Saint-Jean pour leur soutien indéfectible.

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# herb sp	number of herbaceous species
ANAER_N	anaerobic N mineralization rate
ABI	*Abies balsamea*
APP_SP	*Aporrectodea sp.*
ARA	*Aralia nudicaulis*
ARU	*Acer rubrum*
ASA	*Acer saccharum*
ASP	*Acer spicatum*
BR_min	basal respiration in mineral soil layer
BR_org	basal respiration in organic soil layer
CAR	*Carex sp*
comp2_soil	soil PCA scores for axis 2
DAG	direct acyclic diagram
DENDRO	*Dendrobaena octaedra*
DENIT_min	potential dentrification rate in mineral soil layer
DIE	*Diervilla lonicera*
dom_sp	number of species in canopy
DRY	*Dryopteris intermedia*
ERY	*Erythronium americanum*
FF depth	forest floor depth (F layer)
log_earthworms	log of total number of anecic and endogeic earthworms
LUM_RUB	*Lumbricus rubellus*
LUM_SP	*Lumbricus sp.*
LUM_TERR	*Lumbricus terrestris*
MAI	*Maienthemum canadensis*
MB_min	microbial biomass in mineral soil layer
MB_org	microbial biomass in organic soil layer
MLM	Maximum likelihood method
NH4_org	Total ammonium in mineral soil layer
NHH_min	Total ammonium in organic soil layer
NO_ID	juvenile with no clitellum
NO3_all	total nitrate for organic and mineral layers
OXA	*Oxalis stricta*
SÉPAQ	Société des établissements de plein air du Québec
SOR	*Sorbus sp.*
sp.	Species

THU	*Thuya occidentalis*
TRI	*Trientalis borealis*
VIB	*Viburnum alnifolium*

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CHAPITRE 1
INTRODUCTION GÉNÉRALE

Mise en contexte

Dans le présent contexte de mondialisation, les introductions d'espèces exotiques sont favorisées par les échanges de marchandises fréquents entre les continents (Van Driesch 2000). De plus, les forêts nordiques d'Amérique du Nord sont soumises au stress engendré par les changements climatiques et deviennent davantage vulnérables aux invasions biologiques (Bohlen et al. 2004; Aber et al. 2001). Pourtant, en raison de la période de récession économique actuelle, on observe un affaiblissement de la législation gouvernementale en ce qui a trait à la protection du patrimoine naturel (Clap et al. 2009).

Les invasions biologiques peuvent entraîner des modifications majeures au niveau du fonctionnement des écosystèmes. On estime à 120 milliards de dollars le coût en dommages et pertes causés annuellement par les espèces exotiques envahissantes aux États-Unis (Pimentel et al. 2005). Par exemple, les moules zébrées (*Dreissena polymorpha)* et quaggas (*Dreissena bugensis)* causent des dommages estimés à 1 milliard de dollars en se fixant dans les conduites d'eau des usines. Les espèces exotiques envahissantes ont pour caractéristique de modifier la disponibilité des ressources dans l'écosystème, et ce, souvent au détriment des espèces indigènes (Vitousek et al, 1990; Ehrenfeld et al, 2010). Certaines espèces exotiques ont même la capacité de modifier les caractéristiques abiotiques de l'écosystème. Par exemple, dans le sol des États-Unis, les fourmis de feu (*Solenopsis invicta*) et les Lumbricidae au Minnesota sont considérés comme des ingénieurs de l'écosystème, car ils redistribuent la matière organique dans les divers horizons du sol, créant ce que l'on appelle des bioturbations (DeFauw et al. 2008; Ehrenfeld et al, 2010).

1.1 *Histoire des vers de terre en Amérique du Nord*

Même si les gens l'ignorent bien souvent, les vers de terre retrouvés au Québec sont des espèces exotiques. Au Canada, l'ère glaciaire du Wisconsin, qui s'est terminée il y a environ 12 000 ans, a été fatale à presque toutes les espèces indigènes de lombric, excepté celles retrouvées sur la côte du Pacifique (McKey-Fender et al. 1994). Ainsi, en forêt québécoise, les sols, la végétation et les processus de cyclage des nutriments se sont développées en absence de vers de terre (Addison, 2009). Depuis les colonisations européennes du 16_e siècle, plusieurs espèces de Lumbricidae exotiques ont été introduites (Gates, 1982). Étant donné leur dispersion plutôt lente, soit un maximum de cinq à dix mètres par an dans une terre arable, leur migration naturelle ne pourrait pas à elle seule expliquer leur distribution actuelle (Marinissen et al, 1992). Il est donc généralement accepté que les vecteurs de propagation tels que la circulation routière, l'agriculture et le transport de déchets horticoles sont des mécanismes responsables de la colonisation des vers de terre dans les régions nordiques (Hendrix & Bohlen, 2002; Cameron et al, 2007). De plus, étant donné l'absence de compétiteurs, la colonisation des vers de terre exotiques est grandement facilitée (Reynolds, 1973).

1.1.1. *L'écologie des vers de terre*

L'impact des vers de terre sur leur milieu varie en fonction des espèces. Dans son article de 1977, Bouché a divisé les vers de terre en trois groupes écologiques distincts : les vers épigéiques, les vers endogéiques et les vers anéciques.

- Les vers épigéiques sont ceux qui vivent et se nourrissent en surface dans la couche de matière organique. Ils sont détritivores et uniformisent les premiers horizons du sol en les

mélangeant. Ils sont les vers de terre les plus mobiles et ont le taux de reproduction le plus élevé ce qui en fait d'excellents colonisateurs.

- Les vers endogéiques vivent en surface de l'horizon minéral. Ils sont considérés comment étant des géophages, c'est-à-dire qu'ils ingurgitent une grande quantité de matière minérale. Ils mélangent la partie supérieure de la couche minérale avec la partie inférieure de la couche organique.
- Les vers anéciques, les plus gros vers retrouvés au Québec, vivent dans des tunnels verticaux de un à deux mètres de profondeur qu'ils creusent eux-mêmes. Ils sont détritivores et sont responsables des enfouissements de matière organique à de grandes profondeurs, puisqu'ils se bâtissent des garde-manger dans leurs galeries.

Étant donné leurs modes de vie diversifiés, les groupes écologiques de vers de terre ont tous des impacts différents sur le sol. Plus la richesse d'espèces est importante, plus on retrouve de groupes écologiques et plus les impacts sur le sol risquent d'être importants (Addison, 2009). Par exemple, dans le livre de Sonja Migge publié en 2001, il est expliqué que la combinaison de vers de terre anéciques et endogéiques influence davantage la composition des horizons du sol que la présence unique de l'un ou l'autre de ces types écologique. Enfin, les invasions de vers de terre ont le potentiel de se manifester par une succession d'espèces. Ainsi, l'invasion du milieu par les espèces épigéiques facilitera la venue des autres espèces pouvant entraîner à plus long terme une perturbation du milieu plus importante (Frelich et al, 2006). Il a déjà été mentionné dans des études sur la biologie des vers de terre que ceux-ci (à quelques exceptions) étaient aux sols acides et ne les colonisaient pas les zones ayant un pH inférieur à 5 (Tiunov *et al.* 2006). Or, certaines espèces ont été retrouvés au Québec, dans des des pinèdes aux pH inférieurs à 4 (Moore et al, 2003).

1.1.2. *Impacts des vers de terre sur les propriétés du sol*

Les études réalisées sur les sols agricoles rapportent que les vers favorisent la fertilité du sol en plus d'augmenter la porosité du sol, facilitant l'infiltration de l'eau (Satchell 1983; Lee 1985; Edwards and Bohlen 1996; Lavelle et al. 1999). De manière générale, ces études rapportent des effets positifs associés à la présence des vers de terre (Baker et al, 2006).

Dans les 20 dernières années, les chercheurs ont commencé à s'intéresser aux impacts des invasions de vers de terre en milieu forestier. Puisque ces sols ont des propriétés différentes (structure, compaction), les impacts des vers de terre ne sont pas toujours les mêmes que ceux observés dans les sols agricoles. Les vers de terre exotiques vont, de par leurs terriers et leurs excrétions riches en matière labile, modifier les propriétés chimiques et physiques du sol (Lee, 1985; Lavelle, 1988; Bouché, 1977). Ils accélèrent le « cyclage » de l'azote et du carbone en modifiant la structure des communautés microbiennes, c'est-à-dire en augmentant le ratio bactérie : champignons puisque ces derniers sont des décomposeurs plus lents (Scheu & Parkinson, 1994; Groffman et al, 2004; McLean et al, 2006). Ils peuvent diminuer et/ou modifier la colonisation des racines par les mycorhizes (Lawrence et al. 2003; Frelich et al. 2006; Dempsey et al. 2011) qui jouent pourtant un rôle majeur dans l'acquisition de nutriments par les plantes (Perez-Moreno and Read 2000; Brundrett 1988). De plus, ils redistribuent la matière organique dans les couches plus profondes du sol, réduisant parfois la quantité de carbone et le ratio C:N en surface (Alban & Berry, 1994; Bohlen et al, 2004; Suarez et al, 2006). Ils peuvent entraîner une nette diminution de l'épaisseur de la litière présente au sol (Suarez et al, 2006). Par ces perturbations, les vers diminuent la rétention de l'azote dans les couches organiques du sol, mais l'augmente dans les couches plus profondes (Ewing et al 2014). Ces modifications perturbent le cycle de l'azote dans le sol, celui-ci étant plus facilement lessivé, provoquant des pertes pour l'écosystème (Bohlen et al. 2004).

En milieu agricole, de nombreuses études ont déjà révélé que les vers de terre augmentent la production d'oxyde nitreux en créant des macroaggrégats et en enfouissant de la matière

organique en zone anoxique (e.g. Giannopoulos et al. 2009; Kuiper et al. 2013; Marhan et al. 2015), un gaz à effet de serre avec un potentiel de réchauffement global 300 fois plus important que le dioxyde de carbone (IPCC 2013). En comparaison, une seule étude semblable rapporte l'effet des vers de terre sur la production du N2O en milieu forestier. (Burtelow et al. 1998). Cette étude portait uniquement sur des vers de la famille des Megascolecidae. Cependant, une autre étude portant sur le contenu des tubes digestifs des Lumbricidae récoltés en milieu forestier a permis d'observé jusqu'à 256 fois plus de bactéries dénitrifiantes dans leurs milieu intestinal que dans le sol forestier environnant (Karsten et Drake 1997). Une étude permettant de vérifier l'impact des Lumbricidae sur la production de N_2O en milieu forestier était donc la prochaine étape nécessaire à une meilleure compréhension.

1.1.3 *Impact des vers de terre en milieu forestier*

L'introduction de vers de terre exotiques semble mener à un déclin de la diversité et de l'abondance des plantes herbacées de sous-bois (Bohlen et al, 2004; Hale et al, 2006; Holdsworth et al, 2007) et à une diminution de la survie des semis (Hale et al, 2005; Frelish et al, 2006; Holdsworth et al, 2007; Corio et al, 2009; Drouin et al, 2014). Une étude réalisée spécifiquement dans une érablière de l'état de New York a d'ailleurs révélé une diminution de la quantité de semis d'érable à sucre en présence d'une forte abondance de vers de terre (Hale et al, 2006). Les explications proposées pour ces diminutions de couvert et de diversité de sous-étage sont les suivantes : une altération de la composition de la banque de graine en modifiant les conditions de germination, une réduction de la survie des graines lors du passage dans le tractus intestinal des vers ou encore une augmentation de l'herbivorie en absence d'une couche de litière permettant de camoufler les jeunes pousses (Bohlen et al, 2004; Eisenhauer et al, 2009; Drouin 2014). Il a aussi été observé que la quantité de biomasse de racines fines diminuait en présence d'une grande quantité de vers de terre (Fisk et al, 2004).

Les modélisations réalisées par Edward (2004) révèlent que les modifications des propriétés du sol causées par les vers de terre pourraient s'étendre sur une période de deux cents ans le temps que la population de vers de terre se stabilise en des densités plus réalistes selon la capacité de soutien de l'environnement. Dans ce contexte, il s'annonce important de mieux comprendre les impacts que ces modifications pourraient avoir sur la régénération forestière. En effet, pour une période aussi longue, il se pourrait que la composition des forêts subisse des modifications importantes et qu'un retour en arrière soit impossible. Ainsi, des espèces présentement dominantes pourraient subitement être défavorisées par la présence des vers de terre et leur régénération entravée sur le long terme.

Ainsi, avoir une meilleure compréhension de l'influence des vers de terre sur la régénération forestière et le cycle de l'azote serait important afin de guider de futurs plans de conservation dans un contexte où les ressources pour la protection de l'environnement sont fortement limitées. De plus, afin de faciliter les efforts mis sur le contrôle des populations de vers de terre exotiques, une meilleure compréhension, des mécanismes d'introduction est essentielle. Voilà le contexte qui a mené à l'établissement de mon projet de recherche en partenariat avec la SÉPAQ du parc national du Mont-Tremblant. En effet, cet organisme ayant pour but d'assurer la préservation à l'état naturel de la forêt dans une aire de près de 1500 km^2 voulait obtenir davantage de renseignements quant à la propagation des vers exotique sur son territoire. Également, ils étaient intéressés par une étude des impacts potentiels des vers de terre sur le sol et la végétation afin de mieux orienter leur plan de gestion (considérant que jusqu'à présent ils ne considéraient pas du tout les vers de terre exotiques comme une menace potentielle et les vendaient même à l'entrée du parc).

1.2 *Objectifs du projet*

Le premier objectif de notre étude était de décrire la répartition spatiale des vers de terre exotiques en fonction de la circulation routière et la pêche sportive au sein du parc national du Mont-Tremblant. Pour cette première partie, nous avons recensés les populations de vers de terre autour de 3 catégories de lacs (*i.e.* près des routes avec pêches; près des routes sans pêche; loin des routes sans pêche). La deuxième partie de notre projet se concentrait sur certains impacts écologiques des vers de terre exotiques en milieu forestier. Nos objectifs spécifiques étaient : i) Déterminer les impacts des vers de terre sur certaines propriétés dynamiques du sol, et ii) Évaluer l'impact des vers de terre exotiques sur la richesse et la composition du sous-bois forestier (herbacées et régénération ligneuse). Pour ce faire, le pourtour de deux lacs fortement envahis (préalablement identifiés grâce à la première partie de l'étude) a été échantillonné à fine échelle dans l'espoir de détecter des corrélations entre les densités de vers de terre, les propriétés du sol et la diversité végétale.

1.2.1 Hypothèses et prédictions

i) La densité de vers de terre sera corrélée positivement avec l'activité humaine.

Tableau 1. Description des différentes conditions de l'étude et de la richesse / densité hypothétique de vers de terre

Caractéristiques du lac	Sans pêche et sans route	Sans pêche, avec route	Avec pêche et route
Vers de terre	Faible richesse et densité	Richesse et densité moyenne	Richesse et densité élevée

ii) La densité de vers de terre sera corrélée positivement à la production d'oxyde nitreux du sol en milieu forestier.

iii) La richesse et la densité des espèces végétales de sous-bois seront corrélées négativement à la densité et à la richesse des vers de terre.

iv) La présence des vers de terre aura un effet bénéfique pour certaines plantes et un effet négatif pour d'autres, modifiant ainsi la composition végétale de sous-bois.

CHAPITRE 2

EXOTIC EARTHWORMS DISPERSION THROUGH PROTECTED AREAS AND THEIR IMPACTS ON NITROUS OXYDE PRODUCTION

M. Fugère, R. L. Bradley and M. Vellend

2.0. Mise en contexte et contribution des auteurs

Il est généralement accepté que l'être humain est le vecteur principal de dispersion des vers de terre exotiques en Amérique du Nord. Cependant, nous en connaissons peu sur l'effet des diverses activités humaines sur l'abondance et l'assemblage des communautés de Lumbricidae en milieu forestier. De plus, l'effet des Lumbricidae exotiques sur les cycles biogéochimiques en milieu forestier demeure peu connu. Ce chapitre avait pour but de répondre à deux objectifs. D'abord de comparer l'effet des routes carrossables et de la pêche sur l'abondance et la structure des communautés de Lumbricidae en forêt tempérée nordique. Ensuite de vérifier si les vers de terres exotiques auraient le potentiel d'augmenter la production d'oxyde nitreux, un important gaz à effet de serre, en milieu forestier. Nous avons réalisé un échantillonnage autours de 61 lacs du Parc national du Mont-Tremblant, dont 23 situés près d'une route et fortement péchés, 18 situés loin d'une route et non péchés, et 20 situés près d'une route mais non (ou peu) péchés.

Nos résultats révèlent que la pêche et la proximité des routes ont tous les deux un effet positif sur l'abondance des vers de terres. Cependant, l'effet de la pêche est plus grand que l'effet des routes, et cet effet augmente avec le pH du sol. De plus, la pêche est corrélée avec l'abondance de *Lumbricus terrestris* dont le mode de vie est anécique. Nos tests en laboratoire ont révélé que l'abondance de *Lumbricus terrestris* est corrélée positivement avec les taux de nitrification et de dénitrification dans le sol, deux processus pouvant émettre de l'oxyde

nitreux vers l'atmosphère. À notre connaissance, cette étude est la première à faire état d'un lien possible entre la pêche sportive et les émissions de gaz à effet de serre en milieu forestier.

L'article sera prochainement soumis au journal Forest Ecology and Management. La première auteure Martine Fugère a participé à l'élaboration du protocole de terrain, la prise de données, les analyses en laboratoire, les analyses statistiques et la rédaction sous la supervision de son directeur de recherche le professeur Robert Bradley (Université de Sherbrooke) ainsi que son co-directeur le professeur Mark Vellend (Université de Sherbrooke). Ce projet a été réalisé grâce à une collaboration étroite avec la SÉPAQ du Mont-Tremblant.

2.1. Abstract

It is generally accepted that human activities are the main agents of dispersal of exotic earthworms in northeastern North America. However, we know little about the effects of human activities on the abundance and assembly of lumbricid communities in protected areas. The effect of exotic Lumbricidae on biogeochemical cycles in forests also remains unknown. Our first objective was to compare the effects of sport fishing and the proximity of roads on the abundance and structure of Lumbricidae communities in northern temperate forests. Our second objective was to test whether earthworms could potentially increase the production of nitrous oxide, an important greenhouse gas. We sampled earthworm communities around 61 lakes in Mont-Tremblant National Park (Quebec), which included 23 heavily-fished lakes and 20 non-fished lakes located near roads, as well as 18 non-fished lakes that were located in remote areas of the park. Our results revealed that fishing and the proximity to roads both had a positive effect on the abundance of earthworms. The effect of fishing, however, was significantly greater than the effect of road proximity, and this effect increased with increasing soil pH. In addition, fishing was positively correlated with the abundance of *Lumbricus terrestris*, an anecic earthworm species. To assess earthworm impacts on nitrous oxide production, we sampled 47 plots around two heavily invaded lakes. Laboratory soil tests

followed by exploratory redundancy analysis and subsequently by a path analysis revealed that the abundance of *Lumbricus terrestris* reduced soil C:N ratio, which increased soil nitrification and denitrification, two processes that can emit nitrous oxide to the atmosphere. To our knowledge, the present study is the first to reveal a link between recreational fishing and greenhouse gas emissions from forest soils.

Keywords: exotic earthworms, fishing activity, greenhouse gases, protected area, human activity

2.2. Introduction

It is generally recognized that earthworms in eastern North America did not survive the Wisconsin glaciation, which receded about 10,000 years ago (McKey-Fender *et al.* 1994). Consequently earthworm species currently found in this region were introduced by European settlers over the course of recent centuries (Gates 1982). Today, most agricultural fields in southern Quebec are inhabited by exotic earthworm species, the effect of which have been amply described with respect to on nutrient cycling (Eriksen-Hamel *et al.* 2007) and soil structure (Blouin *et al.* 2013). In recent decades, earthworms have also been reported in forest ecosystems that are located north of Quebec's agricultural belt as well as in forest ecosystems in neighbouring regions of Canada and the USA (McLean and Parkinson 1997; Burtelow *et al.* 1998; Moore *et al.* 2009). This has generated interest in the factors driving the dispersal of earthworms into northern temperate forests (Sackett *et al.* 2012), as well as their impacts on the integrity of these ecosystems (Hale *et al.* 2006; Drouin *et al.* 2014; Groffman *et al.* 2015).

Marinissen *et al.* (1992) estimated that the natural rate of earthworm dispersal does not exceed 5–10 m yr^{-1}. It is thus likely that earthworms are introduced into new habitats through human activities (Hale 2008) rather than by natural dispersal. For example, people voluntarily introduce earthworms into gardens to improve compost quality (Edward and Bohlen 1996), or

into polluted sites as agents of soil restoration (Butt 1995). Humans are also responsible for involuntary introductions, for example, with the importation of soil-containing material for agricultural purposes (Hendrix and Bohlen 2002). In northern temperate forests of eastern North America, it is believed that voluntary introductions may result from live unused fish bait being discarded near boat launches and in adjacent areas (Cameron *et al.* 2007; Keller *et al.* 2007; Kilian *et al.* 2012). Involuntary introductions, on the other hand, may occur when earthworms and earthworm cocoons become lodged in tire treads and are later released along roadsides in wooded areas (Cameron *et al.* 2008). Little is known, however, about the relative importance of these two mechanisms in contributing to the abundance and diversity of earthworms in forest ecosystems.

Earthworms are known to affect forest soil properties in many ways. Their feeding activities involve comminution of leaf litter that accelerates its decomposition, thereby causing the loss of the forest floor (Alban and Berry 1994; Suarez *et al.* 2006), a change of soil microbial community structure and function (Li *et al.* 2002; McLean *et al.* 2006), and lower retention of key soil nutrients (Bohlen *et al.* 2004; Sackett *et al.* 2013; Resner *et al.* 2014; Ewing *et al.* 2014). Yet ittle is known about the direct and indirect effects of exotic earthworm communities on the emission of greenhouse gases from forest soils. In agricultural soils, earthworms are reported to stimulate nitrous oxide (N_2O) emissions (Giannopoulos *et al.* 2009; Kuiper *et al.* 2013; Marhan *et al.* 2015), which has a global warming potential 300 times higher than CO_2 (IPCC 2013). Earthworms are known to exacerbate N_2O emissions in agricultural soils via their stimulatory effects on two processes, nitrification and denitrification (Bremner 1997; Wrage *et al.* 2001; Kool *et al.* 2011). Their burrowing activities may increase nitrification by aerating the soil and stimulating microbial growth, resulting in a positive effect on ammonium oxidation (Edward 1995; Blair 1997). They may also enhance denitrification, as their guts and casts are hot-spots for anaerobic denitrifying bacteria (Horn 2003, Drake *et al.* 2007). However, it is uncertain whether nitrification, and by extension denitrification, would be stimulated by earthworms in forest soils. The lower soil pH (Paavolainen et al 1998), the presence of plant derived inhibitory metabolites (Clein and Schimel 1995) and the lack of

fertilizer N may all preclude the open cycling of mineral N and the possibility of N_2O emissions.

Here, we report on a study in which we extensively surveyed earthworm communities along roadsides and near lakes that had been either open or closed to recreational fishing. We quantified and tested the statistical significance of the presence of road and of recreational fishing in models predicting earthworm abundance and community composition. We hypothesized that areas near boat launches around lakes with recreational fishing would constitute hot-spots for exotic earthworms, especially *Lumbricus terrestris*, which is preferred bait species for anglers (Kilian *et al.* 2012). In order to assess exotic earthworms' impacts on soil properties, including N cycling, we intensively sampled earthworm communities and measured soil properties related to microbial biomass and N cycling, including potential nitrification and denitrification, in a sampling grid around two lakes with recreational fishing.

2.3. Materials and methods

2.3.1. *Study site*

The study was conducted within the boundaries of Mont-Tremblant National Park (46°28'39" N, 74°17'26" W), a 1510 km^2 area located ca. 150 km northwest of Montreal (Quebec, Canada). Sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britt.) and balsam fir (*Abies balsamia* [L.] Mill.) are the principal late successional tree species. The park lies at ca. 500 m above sea level within the geological range of the Canadian Shield and contains over 400 lakes distributed in three watersheds. The nearby weather station in Ste-Agathe-des-Monts reports daily average temperatures of -13.1 °C in January and +17.8 °C in July. Average annual rainfall is 1175 mm, of which 31% falls as snow.

The area within the current park boundaries has not had any known permanent human settlements in recent centuries. In the first half of the 20th Century, commercial logging was allowed as well as fishing and hunting by members of 20 exclusive fish-and-game clubs. Public access to a few restricted areas of the Park was granted in 1958, and all logging activities ceased in 1977. Since 1972, the number of daily fishing permits issued for each lake in the Park has been compiled in a registry.

2.3.2. *Experimental design and field sampling*

In summer 2013, we sampled earthworms adjacent to 61 lakes (see figure 1), each surrounded by a narrow belt of conifers near the water's edge, transitioning to mixed hardwood forests moving upslope. Those lakes were selected based on their history of fishing and proximity to roads: (i) 23 lakes were located near roads and had been assigned numerous fishing permits over many years (*i.e.* >3,000 in the past 40 years); (ii) 20 lakes occurred near roads and had seldom (*i.e.* <100) or had never been assigned fishing permits; (iii) 18 lakes occurred in remote areas of the park (*i.e.* at least 500 m from the closest road) and had never been open to recreational fishing. We sampled lakes in random order with respect to this classification, given that earthworm sampling efficiency may change with seasonal soil moisture fluctuations.

At each of the 61 lakes, we established one sampling point in each of four locations: (1) in the coniferous belt near the water's edge, either beside a boat launch or in an area suitable for a boat launch: nearer from the road and easily accessible; (2) 20 m upslope from the first sampling point, in the mixed deciduous forest; (3) 200 m upslope from the first point (*i.e.* perpendicular to the water's edge), also in the mixed deciduous forest; (4) 200 m from the first sampling point, in the coniferous belt near the water's edge. This design allowed us to disentangle the effects of dominant tree type from the effects of proximity to human activities (*i.e.* in the case of lakes located near roads).

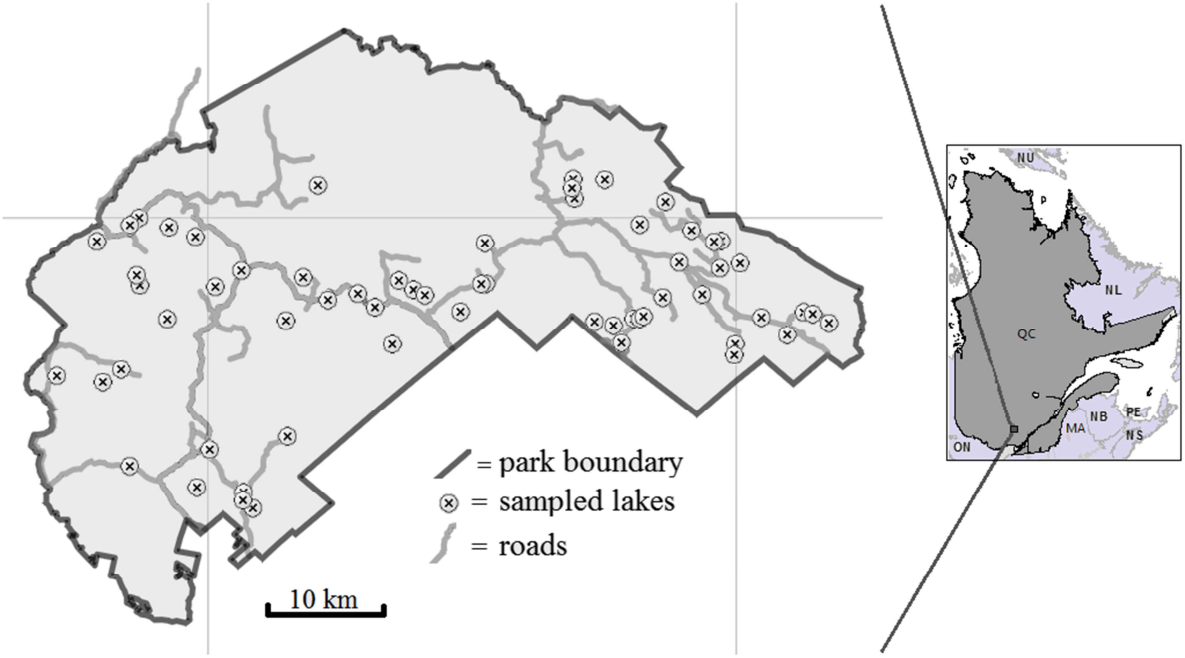


Figure 1: Geographic location of Mont-Tremblant National Park (delimited by dark gray lines). Each circle with an x inside represent a sampled lake (n=61). Pale grey lines represent roads.

At each sampling point, we collected earthworms in four quadrats (25 cm x 25 cm) located at the corners of a 1×1m square area. We removed the litter floor from each quadrat and sifted this material by hand to extract epigeic specimens. We then applied 4 L of water containing 10 g L^{-1} of dry mustard in each quadrat to extract endogeic and anecic species over a 15 min period (Lawrence and Bowers 2002). Finally, we obtained three containers of earthworms sold commercially at the Park's Visitor Center. All earthworms were placed in jars containing 70 % isopropanol and brought back to the laboratory for counting and identification. At each sampling point of each lake, we also collected a bulk sample of organic forest floor F-layer material as well as a bulk sample of the underlying mineral soil up to a depth of 10 cm. Both samples were kept refrigerated until their analysis in laboratory.

In order to evaluate the effects of earthworms on soil microbial and soil N dynamics, we intensively sampled around two lakes in summer 2014. Based on the earthworm survey of 2013, the land adjacent to these two lakes harboured the highest abundance and diversity of earthworms among the 61 lakes, with substantial variation among sampling points. By sampling only two lakes, we removed many site specific sources of error and thus increased the likelihood of observing earthworm mediated effects on soil properties. For each of the two lakes, we established a 30 m x 40 m sampling grid with the first corner of the grid located 20 m upslope from the boat launch and subsequent sampling points occurring at 10 m intervals (*i.e.* 20 sampling points per lake). At each sampling point, we collected a 20 cm x 20 cm sample of organic forest floor F-layer material as well as a sample of the underlying mineral soil up to a depth of 10 cm. Earthworms were then sampled at each sampling point, as described for the extensive earthworm survey of 2013. Soil samples were transported in coolers under ice packs and placed in a cold room (4 °C) until analyzed.

2.3.3. *Earthworm species identification*

Earthworms from both surveys (2013 and 2014) were soaked 24 h in a 10 % formaldehyde solution to fix their tissues and then returned into jars containing isopropanol. Species identification of mature individuals was accomplished under dissecting stereoscope based on the key developed by Reynolds (1977). Juvenile individuals without clitella remained unidentified.

2.3.4. *Soil analyses*

The bulk soil samples from the 2013 survey (*i.e.* 61 lakes, N = 244) were dried at 105 °C to determine moisture content. Soil pH was then measured using a standard hydrogen electrode

(soil:water = 1:10 for organic soil, 1:2 for mineral soil). Total C and N content were determined by high temperature combustion followed by gas analysis using a Vario Macro CN Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The textural class of each mineral soil sample was estimated by a tactile assay developed by Saucier *et al.* (1994), which we first calibrated against the hydrometer method (Bouyoucos1962).

Soil subsamples from the 2 intensively sampled lakes (N = 47) of the 2014 survey were analyzed for moisture content, pH in water, total C and N as well as textural class as described for soil samples from the 2013 survey. Soil basal respiration was measured in field moist soil subsamples by weighing 5 g of organic and 15 g of mineral soil (dry wt. equiv.) into 125 mL sampling vessels. The vessels were covered to minimize moisture loss and left 5 days at room temperature to allow microbial respiration to acclimate. The vessels were then flushed with ambient air for 5 min. and sealed with air-tight lids equipped with rubber septa. Exactly 6 h later, a 2 mL aliquot of gas was taken from the headspace, using a needle and syringe, and injected into a model Varian 431-GC gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with a thermal conductivity detector and He as the carrier gas. CO_2 concentrations were adjusted for fluctuations in room temperature using Ideal Gas Laws and assuming $Q_{10} = 2$.

Soil microbial biomass was measured by substrate induced respiration (SIR, Anderson and Domsch 1978), as modified by Bradley and Fyles (1995). Briefly, field-moist soil samples were removed from the cold room and left 4 h to acclimate to room temperature. Subsamples of organic (5-10 g dry mass equiv.) and mineral (20-25 g dry mass equiv.) soil were then weighed into 125 mL sampling vessels and mixed with 500 mg of a 3% glucose-talc mixture. Sampling vessels were left undisturbed for 100 min. in order to attain maximum initial soil respiration rates. Vessels were then flushed for 5 minutes. with ambient air using an aquarium pump and sealed with air tight lids equipped with septa. After 30 additional minutes, a gas sample was removed from the headspace and analyzed for CO_2 as described above. Substrate induced respiration rates were converted to microbial biomass using equations derived by Anderson and Domsch (1978).

Potential ammonification and nitrification rates were estimated by aerobic soil incubations. Briefly, field moist soil subsamples (15-20 g) were weighed into 500 mL glass jars, covered with polyethylene films to prevent moisture losses while allowing gas exchanges, and left to incubate at room temperature for 30 days. Soils were then extracted in a 1.0 N KCl solution and extracts were analyzed colorimetrically for NH_4^+ and NO_3^- concentrations on a continuous flow analyzer (Astoria-Pacific II, Astoria-Pacific Inc., Clackamas, OR) using respectively the Berthelot and Griess-Ilosvay methods (Mulvaney, 1996).

Potential denitrification rates were measured using the acetylene reduction assay (Ryden *et al.* 1987) Briefly, field moist soil subsamples of organic soil and mineral soil (respectively 5 and 20 g dry mass equiv.) were mixed with 5 mL of KNO_3 solution (500 ppm N) as well as 250 mg of a glucose-talc (1:8) mixture. The treated soil subsamples were then transferred into 125 mL sampling vessels and sealed with airtight lids equipped with rubber septa. The headspace of each sampling vessel was injected with 10 mL of acetylene (C_2H_2) and incubated 18 h at room temperature. A 5 mL aliquot of gas from the headspace of each sampling vessel was then sampled using a needle and syringe, and injected into a model Varian CP 3800 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with an electron-capture detector and He as carrier gas. The measured concentration of ethylene (C_2H_4) in each sample was used as a relative measurement of potential denitrification.

2.3.5. *Statistical analyses*

We first performed a Chi-square test to determine whether the presence or absence of earthworms was distributed randomly across the three categories of lakes sampled in 2013. Subsequently, we tested for the effects of presence or absence of road and fishing activity, soil pH, total C and N, and soil texture on earthworm abundances.

Each sampling point was considered a separate observation, with earthworms abundance summed across quadrats within sampling points. The identity of each lake was included as a random variable in these models to avoid pseudo replication bias. Here we used negative binomial regressions to correct for over-dispersion (*i.e.,* numerous zero values) in earthworm counts across the 61 lakes. We used backward stepwise selection (Harrel 2001) to find the most parsimonious model in which all variables were statistically significant. To have a better understanding of earthworms' distribution within all invaded lakes, we applied a similar modelling approach to the 28 invaded lakes to test the effects of sampling location within lakes and soil variables on earthworm abundances. Tukey HSD tests were used to identify significantly different group means.

In order to explore relationships between earthworm community composition and soil variables, data from the 2014 survey were first analyzed by multivariate redundancy analysis (RDA) with R package "vegan" (van den Wollengerg 1977). We included all our dynamic soil properties from both organic and mineral soil layer: basal respiration, microbial biomass, potential denitrification, anaerobic mineralization and aerobic mineralization. All soil and earthworms variables were standardized. Based on the RDA output (see Results), we further tested for direct and indirect correlations between endogeic + anecic earthworms' density and potential denitrification using Pearson product-moment correlation as well as confirmatory path analysis (Shipley 2000). For our path analysis a sequence of recursive regression models was created including major factors identified in the literature as having a potential impact on denitrification (see Appendix A). A simultaneous test of all independence relationships (Fisher's C test) was performed using the shipley.test function, which indicates if the whole path really could be generating our data. If not, we would retrieve paths that did not fit the independence test. The SEM function in the R package {lavaan}was then used to calculate path coefficients and correlations with direct and indirect effects on the final path. Since variables were not normally distributed, we selected the MLM (Maximum Likelihood Method) estimator (Shipley 2002) for more robust standard error estimations. All analyses were conducted in R3.2.0 statistical software (R Development Core Team, 2015)).

2.4. Results

For the extensive survey conducted in 2013, the presence/absence of earthworms was not distributed randomly across the three categories of lake ($\chi^2 = 8.58$, $P = 0.015$). At remote lakes without fishing, only a single earthworm specimen of a rare species, *Dendrodrilus rubidus*, was found at one site; we detected no earthworms at the 17 remaining lakes in this category. For the other two categories (both near roads), earthworms were 7.3 times more abundant at heavily fished than at lakes with few fishing permits (Fig. 2).

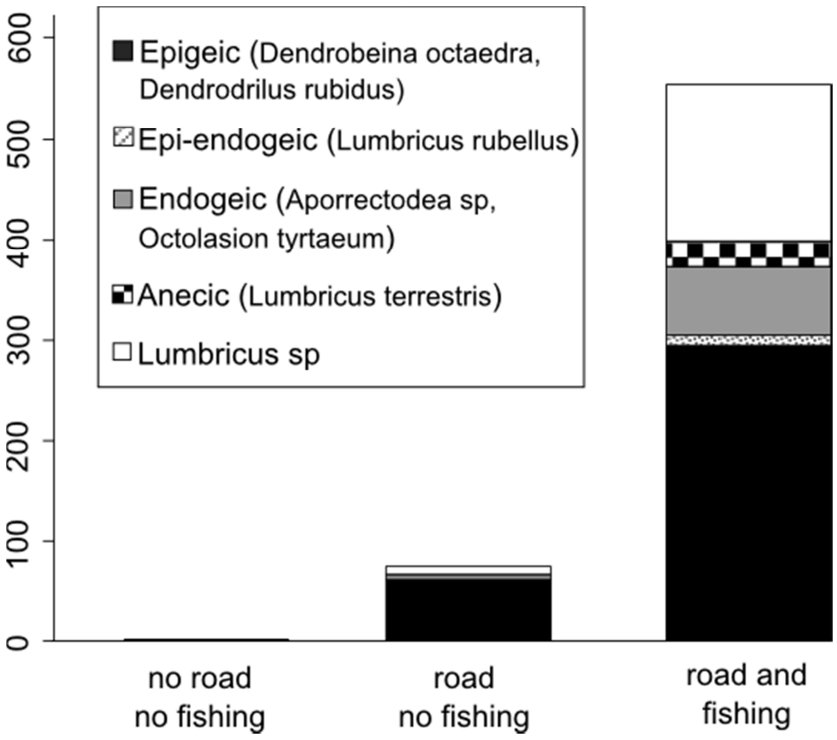


Figure 2: Stacked bar plot showing the total number of each earthworm species recorded within each category of lakes.

Furthermore, nearly 50% of earthworms near lakes with fishing permits were classified as either endogeic or anecic, whereas the epigeic species *Dendrobaena octaedra* comprised about 85% of earthworms around lakes near roads without fishing permits. There was a greater

richness of species around lakes with fishing activity. All earthworms sold at the Park's Visitor Center were of the anecic species *L. terrestris*.

Negative binomial regressions revealed that the number of fishing permits per lake significantly (P = 0.028) increased earthworm abundance, along with soil pH (P < 0.001). When analyzing only the 28 lakes where earthworms were found, negative binomial regressions revealed a significant (P < 0.001) effect of sampling location (distance to boat launch) on earthworm abundances. A *post hoc* Tukey test revealed that earthworms were more abundant near the boat launch, either under conifer or deciduous forest cover, than at 200 m away from the boat launch, either perpendicular or parallel to the shoreline (Fig. 3).

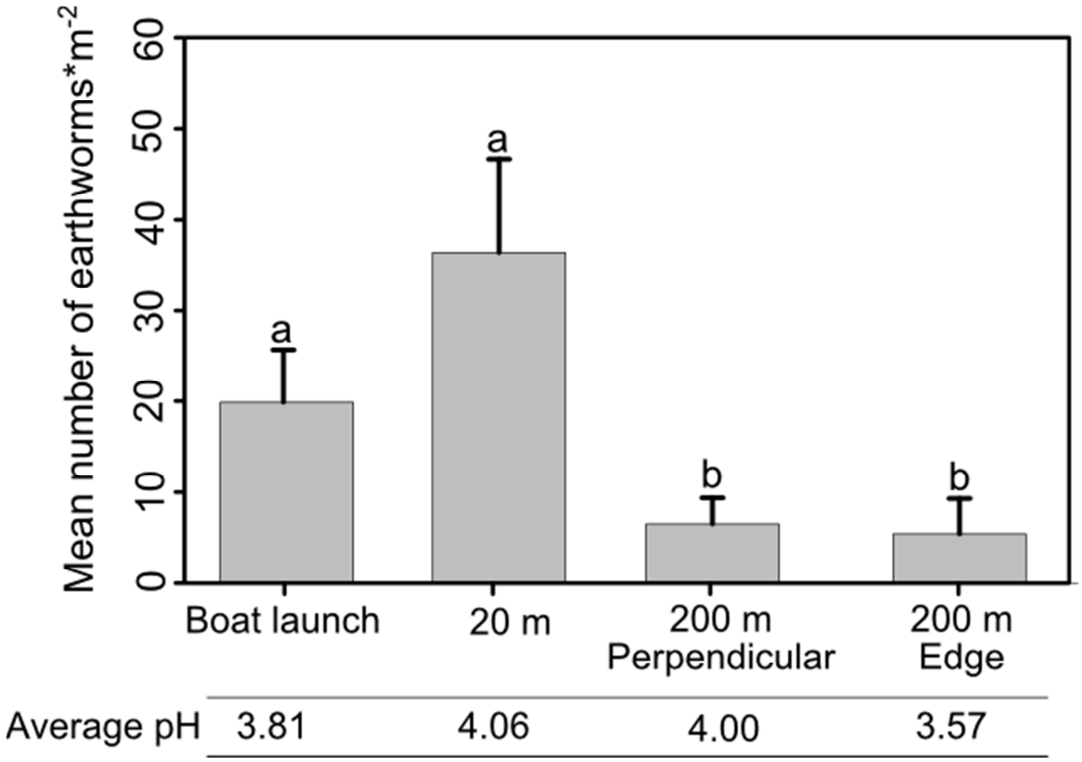


Figure 3: Mean number of earthworms (m-2) and average soil pH at each sampling location within the 28 lakes where earthworms were found. Sampling locations are (1) beside boat launch under coniferous canopy, (2) 20 m upslope from boat launch under mixed deciduous canopy, (3) 200 m upslope from boat launch, under mixed deciduous canopy, (4) 200 m from boat launch near water's edge, under coniferous canopy. Bars with different lower case letters differ significantly (P < 0.05) according to a Tukey test. Vertical lines = 1 S.E.

For the intensive survey conducted in 2014, redundancy analysis (RDA) revealed that earthworms explained 16 % of the variance in dynamic soil properties along the first 2 principal component axes (Fig. 4).

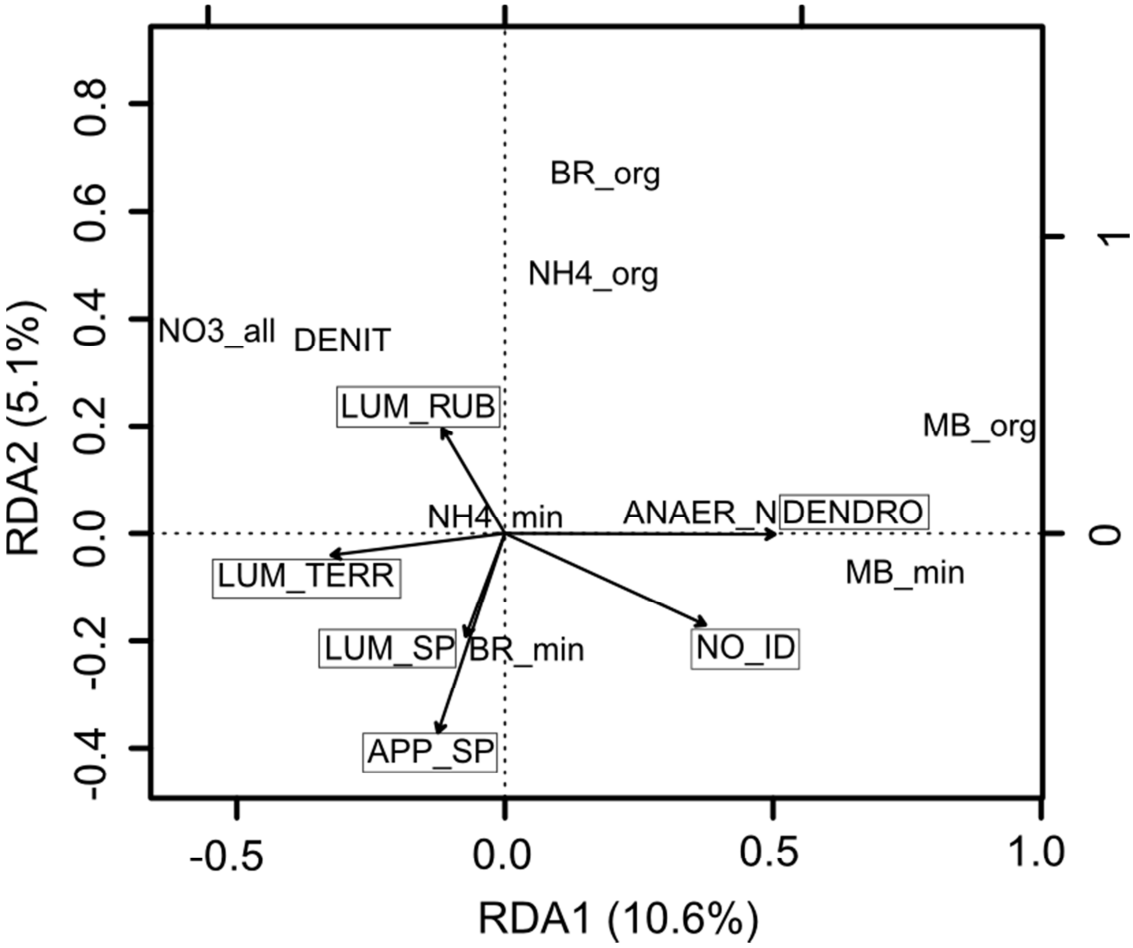


Figure 4: Ordination biplot resulting from redundancy analysis of soil environmental variables (dependent variables with standardized data) in the earthworm species abundancies matrix (independent variables also standardized). Earthworm species are in rectangles: LUM_RUB = *Lumbricus rubellus*; DENDRO = *Dendrobaena octaedra*; NO_ID = unidentified juveniles; APP_SP = *Apporectodea* sp.; LUM_SP = *Lumbricus* sp.; LUM_TERR = *Lumbricus terrestris*. Soil properties are: BR_min and BR_org = basal respiration rate in mineral and organic soil layers; MB = microbial biomass in mineral and organic soil layers; NH4_min and NH4_org =aerobic ammonification rate in mineral and organic soil layers; NO3_all = aerobic nitrification in the combined mineral and organic soil layers; DENIT_min = potential denitrification in the mineral soil layer. The first two principal components explained 16 % of the total variance in soil properties.

The RDA bi-plot also revealed a negative correlation between the abundance of the epigeic species *D. octaedra* and the abundance of endogeic or anecic species. Furthermore, endogeic and anecic species correlated positively with net nitrification as well as with potential denitrification rates. Pearson's correlation revealed a slightly non-significant (P = 0.064) correlation between the abundance of anecic specimen and potential denitrification. However, our path analysis found a significant causal pathway between these two variables via the forest floor C/N ratio as well as via nitrification rates in both the forest floor and mineral soil (Fig. 5).

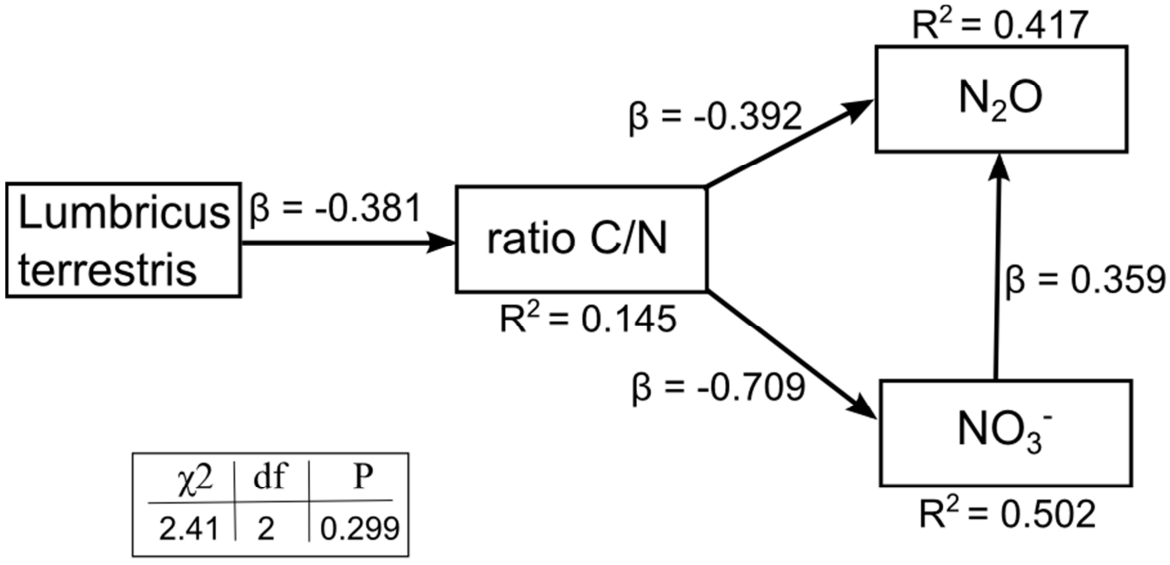


Figure 5: Final path diagram showing the indirect effect of *Lumbricus terrestris* on soil denitrification potential. Path coefficients represent strength (β) and direction of significant correlations. R^2 values are indicated for each endogenous variable.

2.5. Discussion

Our results strongly suggest that dispersal of earthworms into natural mixed-wood forests of Quebec is caused by human activities. Only a single earthworm specimen was found among

the 18 remote lakes that historically had been closed to fishing, and this individual was of a unique and rare species. Our results also indicate that recreational fishing has a greater potential for spreading exotic earthworms in the forests of Mont-Tremblant National Park than does car traffic. Lakes with high levels of recreational fishing had greater earthworm species richness and proportionately more endogeic and anecic species (Fig. 2). *Lumbricus terrestris* was the only species that we detected from bait samples bought at the Park's Visitor Center, and this species was only found adjacent to lakes with frequent fishing. According to several studies (e.g. Holdsworth *et al.* 2007; Corrio *et al.* 2009; Sackett *et al.* 2012), the ecological impacts of endogeic and anecic species, especially *L. terrestris*, are greater than those of epigeic species, such as *D. octaedra*, which was found to be proportionately more abundant near roadside lakes without fishing permits.

Earthworm abundances can be determined both by human-mediated propagule pressure as well as natural variation in soil conditions. Reich *et al.* (2005) sampled earthworms and forest soil properties along a deciduous–coniferous forest gradient and found a correlation between soil pH and earthworm abundances, consistent with the long held view that litter chemistry affects earthworms foraging behaviour and biomass (Piearce 1972; Hendriksen 1990). In our study, however, differences in soil pH under deciduous and coniferous canopies were not as determinant of earthworm abundances as was the proximity to the boat launch (Fig. 3). Given this clustering of earthworms near boat launches, the question remains whether these constitute sink populations or whether they have the potential to spread over time. The risk of exotic earthworms gradually spreading through these forests cannot be dismissed, given that we did find earthworms at 200 m from the boat launches, both along the shorelines and upslope from the water's edge. Also, our survey detected *L. terrestris* around lakes where fishing permits had not been issued for several years prior to our 2013 survey, but had previously been issued numerous fishing permits.

The fact that D. octaedra correlated negatively with all other endogeic and anecic species is consistent with other studies (e.g. Eisenhaur *et al.* 2007). Some have hypothesized (e.g. Pizl *et al.* 1992; Tiunov *et al.* 2006) that this may be due to incompatibilities in these behavioural life

forms, whereby endogeic and anecic species gradually eliminate the forest floor that is vital to the survival of epigeic species. Future earthworm surveys in Parc du Mont-Tremblant could test this hypothesis by verifying whether the introduction of *L. terrestris* by anglers is gradually eliminating *D. octaedra* populations.

Our results provide some clues concerning the nature of interactions between earthworms and microbes, although with only observational data, interpretations are necessarily somewhat speculative. The RDA indicates a positive correlation between the abundance of *D. octaedra* and microbial biomass in the organic forest floor. While some have proposed that epigeic species such as *D. octaedra* might reduce soil microbial biomass (McLean *et al.* 2006), the evidence for such a generalization is lacking. Rather, there are some studies that have shown higher microbial biomass in earthworm casts than in the bulk soil (e.g. Brown 1995; Bohlen *et al.* 2004b). Thus, the positive correlation between *D. octaedra* and microbial biomass could be due to the comminuting activities of this species resulting in the inoculation of forest floor substrates with microbial propagules. It is less obvious why *D. octaedra* correlated positively with microbial biomass in the mineral soil, rather than anecic and endogeic species as others have found (Tiunov and Scheu 1999). On the other hand, microbial basal respiration sorted out along RDA axis 2, suggesting positive effects of *L. rubellus* (epi-endogeic) in the forest floor and of *Aporrectodea sp.* (endogeic) in the mineral soil. That these two species correlated positively with basal respiration implies that they might increase the ratio of bacteria to fungi, as Dempsey *et al.* (2011) have reported. Thus, the primary effect of endogeic and anecic species might be to alter microbial community structure rather than to affect microbial biomass.

We found some intriguing evidence linking earthworms to denitrification, although the exact nature of how earthworms interact with N cycling is potentially quite complex. The global impacts of exotic earthworms on forest ecosystems, notably on soil N cycling, has been reviewed by several authors (e.g., Bohlen *et al.* 2004b; Frelich *et al.* 2006) Our RDA output suggests that *L. terrestris* favors the production of soil NO_3^-, a mobile anion that is prone to be leached to groundwater with excess rainfall. Accordingly, Ewing *et al.* (2015) found that

Northern hardwood forest plots harbouring exotic earthworms had a lower retention of applied ^{15}N-labelled NO_3^- than control plots without earthworms. Whether exotic earthworms increase soil NO_3^- leaching remains controversial, however, as Groffmann *et al.* (2015) recently provided data suggesting that earthworms increase soil available C thereby enhancing microbial N immobilization. Yet the production of NO_3^- is necessary for denitrification to occur, and this process is thought to control N_2O emissions in anaerobic soil microsites (Wrage *et al.* 2001). The mechanism by which earthworms may increase denitrification remains, however, unclear. Some authors report that earthworm biological activity (e.g. feeding, excretion and mucus production) and earthworm structures (e.g. burrows and casts) directly stimulate the reduction of soil NO_3^- (Horn 2003; Drake *et al.* 2007; Costello and Lamberti 2009; Majeed *et al.* 2013). Conversely, Bertora *et al.* (2007) argued that earthworms stimulate N_2O production primarily through indirect effects on soil structure. In our study, we could not establish a direct correlation between the abundance of *L. terrestris* and potential denitrification. Faced with a similar situation, Sackett *et al.* (2012) used exploratory path analysis to reveal causal pathways among measured variables. Accordingly, we found a significant pathway in which *L. terrestris* increases denitrification via its negative effect on soil C:N ratio. In particular, there were two different positive indirect effects of L. terrestris on N20; one indirect path via C:N (-0.381X-0.392) and one via C:N and NO3 (-0.381X-0.709X0.359). There was also a positive direct effect of L. terrestris on N03 via C:N (-0.381X-0.709). This reflects a positive effect of *L. terrestris* on soil organic matter decomposition, which ultimately results in mineral N release and turnover. The fact that *L. terrestris* did not correlate with NH_4^+ production in the RDA biplot is likely due to its positive effect on NH_4^+ oxidation (*i.e.* nitrification), in line with our path diagram (Fig. 5).

2.5.1. *Conclusions and implications*

The global environmental impact of exotic earthworms in North America has prompted some researchers to suggest comprehensive conservation policies that would limit the spread of

these alien species (e.g. Hendrix and Bohlen 2002; Callaham *et al.* 2006). In terms of influencing conservation decisions, studies showing negative impacts of earthworms on forest plant biodiversity (e.g. Hale *et al.* 2006; Drouin *et al.* 2014) probably carry more weight than do data on less tangible and more diffuse impacts of earthworms on biogeochemical cycles. Yet, the chronic effects of earthworms on the cycling of reactive N through the biosphere and geosphere may be more pernicious and irremediable than other impacts. The problem is that biogeochemical cycle alternations are less liable to be detected than are losses of conspicuous plant species. The two salient points that have emerged from our study are (1) recreational fishing is responsible for the introduction of *L. terrestris* in forests ecosystems of Mont-Tremblant National Park, and (2) this exotic earthworm species can lead to faster N cycling with potential increases in NO_3^- leaching and soil N_2O emissions. Future research should strive, therefore, to quantify these fluxes under *in situ* conditions, and scale them up to the landscape scale.

As we have shown, there remain many forested areas in southern Quebec that are earthworm-free (*i.e.*, places distant from roads and free from sport fishing). Protecting such places from the spread of exotic earthworms is important in order to maintain "baseline" conditions permitting evaluation of earthworm impacts. The *Société des Établissements de Plein Air du Québec* (SÉPAQ) is the governing body whose mandate is to preserve the natural heritage of our forests through the management of 24 national parks occupying an area greater than 37,400 km^2. In their mission statement, SÉPAQ states that "*a national park's primary purpose is to ensure the conservation and permanent protection of areas representative of the natural regions of Québec and of natural sites with outstanding features, in particular because of their biological diversity, while providing the public with access to those areas.*" Accordingly, SÉPAQ has established a series of conservation measures, such as forbidding domestic animals such as dogs and cats from entering national parks. Paradoxically, SÉPAQ sells live exotic earthworms to anglers at their visitor centers, thereby contributing substantially to their spread into new habitats. Further work should include direct plans to inform and educate the public on exotic earthworms' issue, and to help develop future policy that would end the spread of exotic earthworms into protected forest areas in North America.

2.6. Acknowledgements

We thank the following SÉPAQ personnel, Patrick Graillon, Hugues Tennier and Émilie Dorion for their kind support regarding site access, field lodging, and information about site history. Funding for this project came from NSERC (Natural Sciences and Engineering Research Council of Canada), FQRNT (Fonds Québécois de la Recherche sur la Nature et Technologies), and SÉPAQ.

2.7. References

Alban, D.H. & Berry, E.C. (1994). Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. *Applied Soil Ecology*, 1, 243–249.

Anderson J. P. E. and Domsch K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215-221.

Bertora, C., van Vliet, P.C.J., Hummelink, E.W.J., van Groenigen, J.W., (2007). Do earthworms increase N2O emissions in ploughed grassland? Soil Biology and Biochemistry 39, 632e640.

Blair, J. M., Parmelee, R. W., Allen, M. F., McCartney, D. A., & Stinner, B. R. (1997). Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. *Soil Biology and Biochemistry*, *29*(3), 361-367.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J. (2013). A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64, 161e182.

Bohlen, P. J., Pelletier, D. M., Groffman, P. M., Fahey, T. J., & Fisk, M. C. (2004). Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests. *Ecosystems*, *7*(1), 13–27

Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson,D. (2004b). Non-native invasive earthworms as agent of change in northern temperate forests. Front. Ecol. Environ. *2*, 427–435.

.

Bouyoucos, G. J., (1962). Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54: 464-465.

Bradley R. and J.W. Fyles. (1995). A kinetic parameter describing soil available carbon and its relationship to rate increase in C mineralization. Soil Biology & Biochemistry 27:167-172.

Bremner, J. M. (1997). Sources of nitrous oxide in soils. *Nutrient cycling in Agroecosystems*, *49*(1-3), 7-16.

Brown, G.G. (1995). How do earthworms affect microfloral and faunal community diversity? Plant Soil 170, 209-231.

Burtelow A.E., Bohlen P.J., Groffman P.M. (1998). Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Appl Soil Ecol 9:197–202

Butt, K. R., Frederickson, J., & Morris, R. M. (1995). An earthworm cultivation and soil inoculation technique for land restoration, *4*, 1–9.

Callaham Jr, M. A., González, G., Hale, C. M., Heneghan, L., Lachnicht, S. L., & Zou, X. (2006). Policy and management responses to earthworm invasions in North America. In Biological Invasions Belowground: Earthworms as Invasive Species p. 117-129.

Cameron, E. K., Bayne, E. M., Landscape, I., & Group, M. (2007). Human-facilitated invasion of exotic earthworms into northern boreal forests 1, *14*(4).

Cameron, E. K., Bayne, E. M., & Coltman, D. W. (2008). Genetic structure of invasive earthworms Dendrobaena octaedra in the boreal forest of Alberta: insights into introduction mechanisms. *Molecular ecology*, *17*(5), 1189–97.

Clein JS, Schimel JP. (1995). Nitrogen turnover and availability during succession from Alder to Poplar in Alaskan taiga. Soil Biology and Biochemistry 27: 743–752

Corio, K., Wolf, A., Draney, M., & Fewless, G. (2009). Exotic earthworms of great lakes forests: A search for indicator plant species in maple forests. *Forest Ecology and Management*, *258*(7), 1059–1066.

Costello, D. M., & Lamberti, G. a. (2009). Biological and physical effects of non-native earthworms on nitrogen cycling in riparian soils. *Soil Biology and Biochemistry*, *41*(10),

2230–2235.

Dempsey, M. a., Fisk, M. C., & Fahey, T. J. (2011). Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. *Soil Biology and Biochemistry*, *43*(10), 2135–2141.

Drake, H.L., Horn, M.A. (2006). Earthworms as a transient heaven for terrestrial denitrifying microbes: a review. Engineering in Life Sciences 6, 261-265
Drouin, M., Bradley, R., Lapointe, L., & Whalen, J. (2014). Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. *Applied Soil Ecology*, *75*, 145–149.

Edwards C.A., Bohlen P. (1995) Biology and ecology of earthworms. Chapman and Hall, New York, 426 pp

Eisenhauer, N., Partsch, S., Parkinson, D., & Scheu, S. (2007). Invasion of a deciduous forest by earthworms: Changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biology and Biochemistry*, *39*(5), 1099–1110.

Eriksen-Hamel, N. S., & Whalen, J. K. (2007). Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems. *Agriculture, Ecosystems & Environment*, *120*(2-4), 442–448.

Ewing, H. a., Tuininga, A. R., Groffman, P. M., Weathers, K. C., Fahey, T. J., Fisk, M. C., Bohlen, P. J., et Suarez, E. (2014). Earthworms Reduce Biotic 15-Nitrogen Retention in Northern Hardwood Forests. *Ecosystems*, *18*(2), 328–342.

Frelich, L. E., Hale, C. M., Scheu, S., Holdsworth, A. R., Heneghan, L., Bohlen, P. J., & Reich, P. B. (2006). Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions*, *8*(6), 1235–1245.

Gates G.E. (1982). Farewell to North American megadriles. Megadrilogica 4:12–77
Giannopoulos, G., Pulleman, M. M., & Van Groenigen, J. W. (2010). Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N2O dynamics in agricultural soil. *Soil Biology and Biochemistry*, *42*(4), 618–625.

Groffman, P. M., Fahey, T. J., Fisk, M. C., Yavitt, J. B., Sherman, R. E., Bohlen, P. J., & Maerz, J. C. (2015). Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. *Soil Biology and Biochemistry*, *87*, 51–58.

Hale, C.M., Frelich, L.E. & Reich, P.B. (2006). Changes in hardwood forest understory plant communities in response to European earthworm invasions.*Ecology*, 87, 1637–1649.

Hale, C. M. (2008). Evidence for human-mediated dispersal of exotic earthworms: support for exploring strategies to limit further spread. Molecular Ecology, 17: 1165-1169.

Harrell, F.E.(2001). Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis. New York: Springer.

Hendriksen NB. (1990). Leaf litter selection by detritivore and geophagous earthworms. Biol Fertil Soils 10:17–21.
Hendrix PF, Bohlen PJ. (2002). Exotic earthworm invasions in North America: Ecological and policy implications. BioScience 52:801–11

Holdsworth, A. R., Frelich, L. E., & Reich, P. B. (2007). Effects of Earthworm Invasion on Plant Species Richness in Northern Hardwood Forests. *Conservation Biology*, *21*(4), 997–1008.

Horn, M. A., Schramm, A., & Drake, H. L. (2003). The Earthworm Gut : an Ideal Habitat for Ingested N 2 O-Producing Microorganisms, *69*(3), 1662–1669.

James, S. W., (1995). Systematics, biogeography and ecology of Nearctic earthworms from eastern, central, southern and southwestern United States. Pages 29-52 in P. F. Hendrix (ed). Earthworm Ecology and Biogeography in North America. CRC Press, Boca Raton, Florida.

Keller, R. P., Cox, A. N., Loon, C. V. A. N., Lodge, D. M., Herborg, L., & Rothlisberger, J. (2006). From Bait Shops to the Forest Floor : Earthworm Use and Disposal by Anglers, (574), 321–328.

Kilian, J. V., Klauda, R. J., Widman, S., Kashiwagi, M., Bourquin, R., Weglein, S., & Schuster, J. (2012). An assessment of a bait industry and angler behavior as a vector of invasive species. *Biological Invasions*, *14*(7), 1469–1481.

Kool, D. M., Van Groenigen, J. W., & Wrage, N. (2011). *Source determination of nitrous oxide based on nitrogen and oxygen isotope tracing dealing with oxygen exchange. Methods in enzymology* 1st ed., Vol. 496, pp. 139–60.

Kuiper, I., de Deyn, G. B., Thakur, M. P., & van Groenigen, J. W. (2013). Soil invertebrate fauna affect N2 O emissions from soil. *Global change biology*, *19*(9), 2814–25.

Lawrence, A. P. & M. P. Bowers, (2002). A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biology & Biochemistry, 34: 549-552.

Li, X., Fisk, M. C., Fahey, T. J., & Bohlen, P. J. (2002). Influence of earthworm invasion on soil microbial biomass and activity in a northern hardwood forest, *34*, 1929–1937.

Majeed, M.Z., Miambi, E., Barois, I., Blanchart, E., Brauman, A., (2013). Emissions of nitrous oxide from casts of tropical earthworms belonging to different ecological categories. Pedobiologia 56, 49-58.

Marhan, S., Auber, J., & Poll, C. (2015). Additive effects of earthworms, nitrogen-rich litter and elevated soil temperature on N2O emission and nitrate leaching from an arable soil. *Applied Soil Ecology*, *86*(3), 55–61.

Marinissen J.C.Y., van den Bosch F. (1992). Colonization of new habitats by earthworms. Oecologia 91:371–376

McKey-Fender D., Fender W.M., Marshall V.G. (1994). North American earthworms native to Vancouver Island and the Olympic Peninsula. Can J Zool 72:1325–1339
McLean, M. a., Migge-Kleian, S., & Parkinson, D. (2006). Earthworm invasions of ecosystems devoid of earthworms: effects on soil microbes. *Biological Invasions*, *8*(6), 1257–1273.

McLean M.A., Parkinson D. (1997). Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of Dendrobaena octaedra (Oligochaeta, Lumbricidae). Soil Biol Biochem 29: 537–540

Moore, J.-D., Ouimet, R., Reynolds, J.W. (2009). Premières mentions de vers de terre dans trois écosystèmes forestiers du Bouclier Canadien. Nat. Can. *133*, 31-37. Piearce, T.G., Roggero, N., Tipping, R. (1994). Earthworms and seeds. J. Biol

Mulvaney, R. L. (1996). Nitrogen – Inorganic forms. Pages 1123-1184 in D. L. Sparks *et al.* (eds.), Methods of Soil Analysis. Part 2. Chemical Properties. Soil Science Society of America Book Series 5. SSSA, Madison, WI.

Paavolainen, L., Smolander, A., (1998). Nitrification and denitrification in soil from a clear-cut norway spruce (Picea abies) stand. Soil Biology & Biochemistry 30, 775±781.
Piearce, T.G., Roggero, N., Tipping, R. (1994). Earthworms and seeds. J. Biol. Educ. 28,195-202.

Pizl, V. (1992). Succession of earthworm populations in abandoned fields. Soil Biol. Biochem. 24, 1623-8.

Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., and Midgley, P. M. (2014). *Climate change 2013: The physical science basis.* T. Stocker (Ed.). Cambridge, UK, and New York: Cambridge University Press, 1535p.

Reich, P.B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M. (2005). Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecol. Lett., 8, 811–818.

Resner, K., Yoo, K., Sebestyen, S. D., Aufdenkampe, A., Hale, C., Lyttle, A., & Blum, A. (2014). Invasive Earthworms Deplete Key Soil Inorganic Nutrients (Ca, Mg, K, and P) in a Northern Hardwood Forest. *Ecosystems*, *18*(1), 89–102.

Reynolds J.W. (1977). The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Life Sci Misc Publ, Royal Ont Mus, ON, Canada

Ryden, J. C., Skinner, J. H., & Nixon, D. J. (1987). Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. *Soil Biology and Biochemistry*, *19*(6), 753-757.

Sackett, T. E., Smith, S. M., & Basiliko, N. (2012). Exotic earthworm distribution in a mixed-use northern temperate forest region : influence of disturbance type , development age , and soils, *381*, 375–381.

Sackett, T. E., Smith, S. M., & Basiliko, N. (2013). Indirect and direct effects of exotic earthworms on soil nutrient and carbon pools in North American temperate forests. *Soil Biology and Biochemistry*, *57*, 459–467.

Saucier, J.-P. (1994). Le point d'observation ecologique : normes techniques. Ministère des Ressources naturelles du Québec, Québec.

Shipley, Bill.(2002). Cause and correlation in biology: a user's guide to path analysis, Cambridge University Press, United Kingdom, 332p.

Suárez, E. R., Fahey, T. J., Yavitt, J. B., Groffman, P. M., & Bohlen, P. J. (2006). Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. *Ecological Applications*, *16*(1), 154-165.

Tiunov, A. V., C. M. Hale, A. R. Holdsworth & T. S. Vsevolodova-Perel, 2006. Invasion patterns of Lumbricidae into the previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. Biological Invasions, 8: 1223-1234.

Tiunov, A. V., & Scheu, S. (2000). Microfungal communities in soil , litter and casts of Lumbricus terrestris L . (Lumbricidae): a laboratory experiment, *14*, 17–26.

Van den Wollenberg, A. L. (1977). Redundancy Analysis-An Alternative for Canonical Correlation Analysis, Psychometrika, 42(June), 207-219

Wrage, N., Velthof, G. L., Beusichem, M. L. V., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide, *33*, 1723–1732.

CHAPITRE 3

EXOTIC EARTHWORMS IMPACTS ON FOREST REGENERATION IN NORTHERN TEMPERATE FOREST

M. Fugère, M. Vellend and R. L. Bradley

3.0. Mise en contexte et contribution des auteurs

Les invasions de vers de terre exotiques sont récemment devenues le sujet de nombreuses inquiétudes quant à leur impact sur les milieux forestiers. Dans les 30 dernières années des études ont montré que les vers de terre modifiaient grandement les propriétés physiques et chimiques des sols envahis, modifiant le cyclage des nutriments et par le fait même la végétation environnante. Dans ce chapitre nous voulions vérifier si ces effets imputables aux vers de terre demeurent, même en contrôlant pour les effets confondants des différentes propriétés statiques du sol. De plus, nous voulions améliorer notre compréhension du phénomène en identifiant les impacts directs et indirects des vers de terre sur la richesse de la strate végétale de sous-bois. Nous avons donc procédé à l'échantillonnage de 47 points dans une zone protégée fortement envahie par les vers de terre (identifiée lors d'une expérience antérieure).. Nous avons pu identifier que les vers influencent bel et bien la végétation et réduisent la richesse en espèces herbacées.

L'article sera soumis sous forme de « short communication » à un journal qui demeure encore à déterminer. La première auteure Martine Fugère a participé à l'élaboration du protocole de terrain, la prise de données, les analyses en laboratoire, les analyses statistiques et la rédaction sous la supervision de son co-directeur de recherche le professeur Mark Vellend (Université de Sherbrooke) ainsi que son directeur le professeur Robert Bradley (Université de Sherbrooke). Ce projet a été réalisé grâce à une collaboration avec la SÉPAQ du Mont-Tremblant.

3.1. Abstract

Over past decades, researchers have tried to understand the impacts of exotic earthworms in newly invaded ecosystems of North America. By doing so, many studies highlighted negative impacts on seedlings and herbaceous species richness and cover density. However, earthworm effects on understory composition are often ambiguous between studies, with no control for confounding bulk soil properties such as pH, C/N ratio, etc. In our study, we tried to assess earthworms' impact on forest understory in a protected area in Québec, Canada. Our first objective was to identify how exotic earthworms affect understory composition when controlling for soil properties that could be acting as confounding factors. Our second objective was to identify direct and indirect impacts of earthworms on seedlings and herbaceous species richness. In September 2013, we surveyed 47 plots around two heavily invaded lakes to assess relationships between earthworm and plant communities. Our results revealed that endogeic and anecic species negatively affect *Trientalis borealis*, *Maienthemum canadense* and *Dryopteris intermedia* and positively affect *Carex sp.*. No major impacts were found on seedling species. The anecic specie *Lumbricus terrestris* was found to have an indirect negative impact on herbaceous species richness through a reduction of the forest floor thickness. Our study revealed that earthworms do have an impact on understory composition when controlling for confounding factors which emphasizes the importance of controlling their expansion in protected areas.

Keywords: exotic earthworms, herbaceous, species richness, forest floor thickness, seedlings, understory composition

3.2. Introduction

Since being introduced by European settlers in the 16th century, exotic earthworms have spread across North America through human activities such as sport fishing (Gates 1982; Hendrix & Bohlen 2002; Cameron et al. 2007). Even if they are usually considered beneficial in agricultural lands (Tomlin 2003; Baker 2006) studies over the past decade have clearly demonstrated negative impacts on newly invaded forest ecosystems (Bohlen et al. 2004; Addison 2009). Earthworms tend to alter soil properties by reducing organic soil horizons, thereby reducing soil water retention and increasing phosphorus and nitrogen leaching (Scheu 2003; Frelich et al. 2006; Hale et al. 2006). They may also modify soil biodiversity, such as reducing the abundance of microarthropods and mycorrhizae (Lawrence et al. 2003; Frelich et al. 2006; Dempsey et al. 2011; Cameron et al. 2013).

Earthworms represent a threat to tree regeneration. They reduce the abundance of fine roots (Fisk et al. 2004) and negatively influence the germination of tree seeds (Drouin et al. 2014), earthworms represent a threat to tree regeneration. However, their impact on understory vegetation more generally remains unclear. Although earthworms are known for reducing the biodiversity of herbaceous species, including seedlings (Hale et al. 2006; Holdsworth et al. 2007) studies of earthworm effects on understory composition have revealed differing and contradictory impacts (Hales et al. 2006; Holdsworth et al. 2007; Corio et al 2009; Nuzzo et al. 2009; Hopfensperger et al. 2011; Davalos et al. 2014). We wanted to assess earthworms' impacts in our specific protected area in Quebec. It was important for our SEPAQ partners to verify if earthworms have a negative impact on some native seedling and herbaceous species.

Most previous studies have investigated correlations between earthworm biomass and vegetation properties, without controlling for confounding factors such as soil properties (e.g., pH, texture, moisture, C/N ratio) that could affect both earthworms and vegetation

simultaneously. In this study, we surveyed earthworms, vegetation, and environmental characteristics in plots adjacent to two heavily invaded lakes in Parc national du Mont-Tremblant, Québec, Canada. Our first expectation was that earthworm densities would be a strong predictor of understory plant species. Our second prediction was that earthworm density would be negatively correlated with understory plant species richness.

3.3. Materials and methods

3.3.1. *Plant and earthworm communities*

In September 2013, we surveyed plots around two heavily invaded lakes to assess relationships between earthworm and plant communities. We chose these two lakes in order to capture small-scale earthworm density gradients in vegetation dominated by the same species, thus avoiding bias from potentially confounding factors (e.g., different histories of disturbance at different lakes). Sampling started near the boat launch in deciduous vegetation and design was made on site to keep sampling points within relatively constant dominant vegetation. We sampled ~20 points at each lake, with each point separated from others by 8-10 meters. Understory vegetation (tree seedlings < 30 cm in height, herbaceous species) was surveyed both in September 2013 and June 2014 (data combined across surveys) by estimating total cover for each species within a 1.75 m radius around each sampling point.

Earthworm density was estimated by sampling four quadrats, each 0.125m2, positioned within a distance of 1.75m from the central point. Litter was first removed and earthworms hand sorted and collected from it, then 2 liters of dry mustard solution (40g/l) was applied in each quadrat(Lawrence and Bowers 2002). All earthworms emerging within 8 minutes were

collected and stored in 70% isopropanol for later identification. In laboratory, earthworms were soaked for 24 h in 10 % aqueous formaldehyde to fix their tissues and then returned into jars containing isopropanol. Species identification of mature individuals was accomplished with a binocular light microscope based on the key that was developed by Reynolds (1977). Juvenile individuals without clitella remained unidentified.

3.3.2. *Potential predictor variables*

As potential predictors of both earthworm and plant community characteristics, we measured the distance from the access road and the boat launch to each point (based on previous results, Fugère *et al*., unpublished). Canopy cover for each tree species was visually estimated in a 4 m radius. Thickness of the forest floor layer and thickness of the litter were measured for each sampling point. Soil from the topmost 5 cm of organic layer and from the mineral layer were sampled by filling 2L Ziploc® bags and placing these in coolers for transport, then in refrigerators, to maintain low temperature and moisture content until laboratory analysis.

On each soil sample we estimated five different properties. Moisture content was estimated gravimetrically by drying sub-samples at 105 ^{0}C for 36 h. Soil pH was then measured using a standard hydrogen electrode (soil: water = 1:10 for organic soil, 1:2 for mineral soil). Total C and N content were determined by high temperature combustion followed by gas analysis using a Vario Macro CN Analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The textural class of mineral soil samples was determined by a standard field protocol (Saucier 1994), which was calibrated with particle size analysis using the hydrometer method (Bouyoucos 1962).

3.3.3. *Statistical analysis*

Our analyses aimed to assess the influence of earthworm community properties on vegetation composition and diversity, while controlling for soil properties. To avoid overfitting our model with too many environmental variables, we first ran a principal components analysis (PCA) to summarize soil properties in fewer axes. The PCA included pH, soil moisture, C/N ratio, forest floor thickness and texture. We extracted three axes (see results for details) and the PCA scores from these axes were integrated into a redundancy analysis (RDA). The RDA also included endogeic and anecic earthworm densities, total earthworm biomass, distances from human activities (road and boat launch) and canopy vegetation richness and total cover as explanatory variables for the composition of herbaceous and tree-seedling communities. All plant and earthworms variables were standardized. A Hellinger transformation was applied to plant abundances. because of the frequent occurrence of zero values. This transformation consisted in dividing the abundance values by the site abundance, and then to squareroot-transform the result. This transformation gave proportionate weight to the rarer plant species (Legendre & Gallagher, 2001). We then realised a permutation test with a stepwise selection on the RDA using the R function ordi2step in {vegan} package (R Development Core Team, 2015) . We identified variables having a significant effect (alpha = 0.05) on plant community composition based on 1000 permutations. A correlation matrix was produced from the final RDA in order to understand which herbaceous and seedlings species were correlated most strongly with earthworm variables.

To test the impact of earthworms on species richness and total cover of the herbaceous and tree-seedling communities, two path analyses were performed. A directed acyclic graph (DAG), a sequence of recursive regression models, was created including major factors identified in the literature as having a potential impact on understory richness (see Appendix A). This DAG included all direct and indirect effects known in between soil properties and vegetation. A simultaneous test of all independence relationships (Fisher's C test) was

performed using the shipley.test function, which indicates if the whole path really could be generating our data. If not, we would retrieve paths that did not fit the independence test. The SEM function in the R package {lavaan}was then used to calculate path coefficients and correlations with direct and indirect effects on the final path. Since variables were not normally distributed, we selected the MLM (Maximum Likelihood Method) estimator (Shipley 2002) for more robust standard error estimations. All analyses were conducted in R3.2.0 statistical software (R Development Core Team, 2015).

3.4. Results

3.4.1. *Earthworms and understory composition*

In the PCA of soil characteristics, the first three axes explained 72% of the total variance. Axis 1 correlated most strongly with C/N ratio and texture, axis 2 with pH and forest floor depth and axis 3 with soil moisture. The subsequent RDA revealeda significant effect of earthworm abundance (endogeic and anecic species combined) on plant community composition (Fig. 1). Even though they were a lot of epigeic (*Dendrobeina octaedra*) specimen on our sites, their presence seems to have little impact on understory vegetation. Based on correlations between endogeic and anecic abundances and individual plant species abundances, earthworms appear to have a negative impact on *Trientalis borealis* (r = -0.35), *Maienthemum canadense* (r = -0.43), and *Dryopteris intermedia* (r = -0.35) and a positive effect on *Carex sp.* (r = 0.51). No major impacts were found on seedling species.

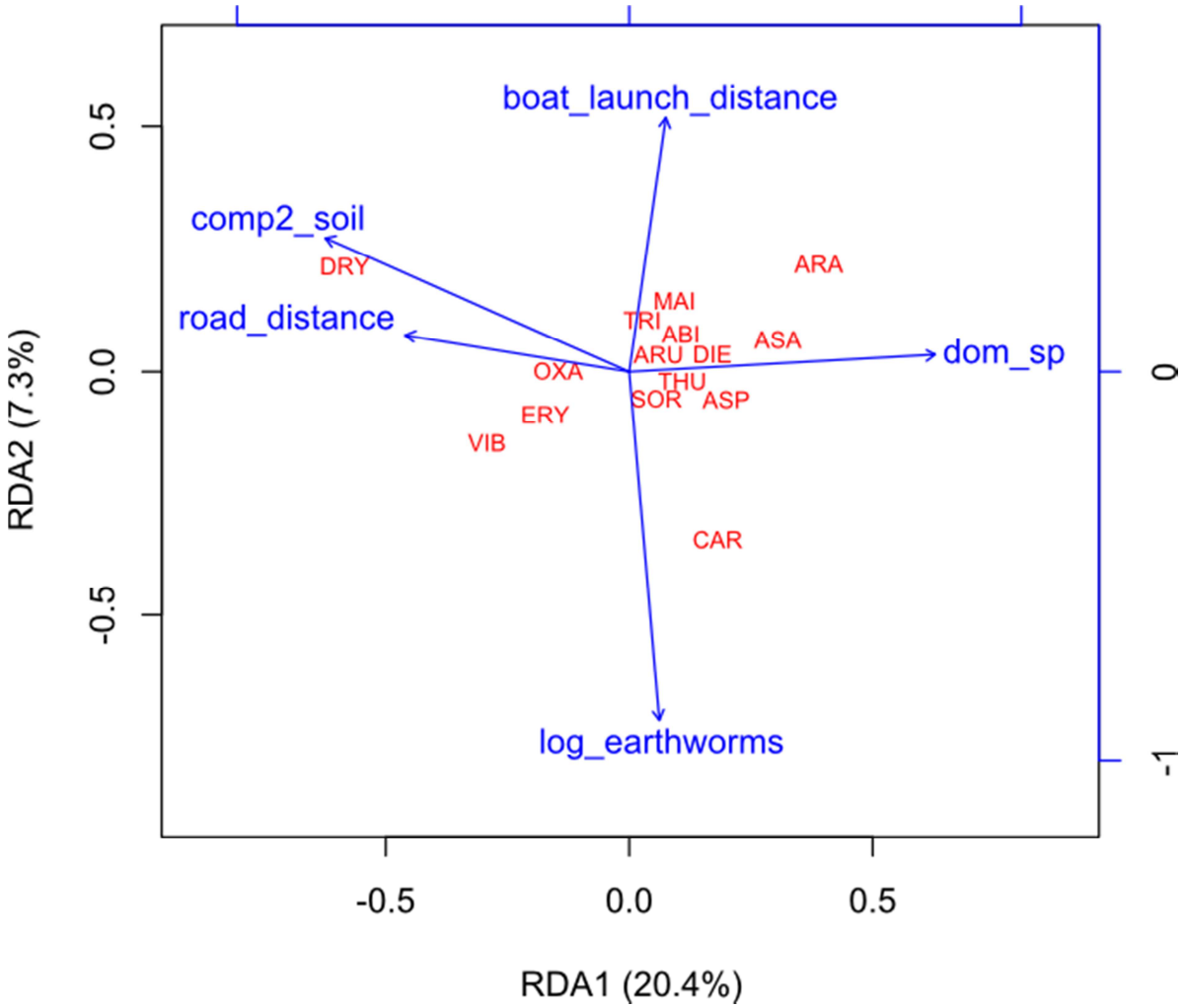


Figure 1: Ordination biplot resulting from redundancy analysis of understory plant species total cover (dependent variables with standardized data) in the environmental matrix (independent variables, also standardized). Environmental variables are: road_distance and boat_launch distance = distance to the nearest road and distance to boat launch; Comp2_soil = second principal component scores from PCA generated with soil variables; dom_sp = dominant tree species within a 4 m radius; log_earthworms = log (anecic + endogeic earthworms).Abbreviations for dependent variables are: CAR = *Carex* sp.; VIB = *Viburnum alnifolium*; ERY = *Erythronium americanum*; OXA = *Oxalis stricta*; DRY = *Dryopteris intermedia*; TRI = *Trientalis borealis*; ARU = *Acer rubrum*; SOR = *Sorbus* sp.; THU = *Thuya occidentalis*; ABI = *Abies balsamea*; MAI = *Maienthemum canadensis*; DIE= *Diervilla lonicera*; ASA = *Acer saccharum*; ARA = *Aralia nudicaulis*; ASP = *Acer spicatum*. The first two principal components explained 28 % of the total variance in understory plant community composition.

3.4.2. *Earthworms and species richness*

Path analyses aiming to predict tree seedling richness and cover showed no significant effects, either direct or indirect, involving earthworms; only canopy richness had a significant effect on seedling richness ($p<0.001$), explaining 29% of its variance.

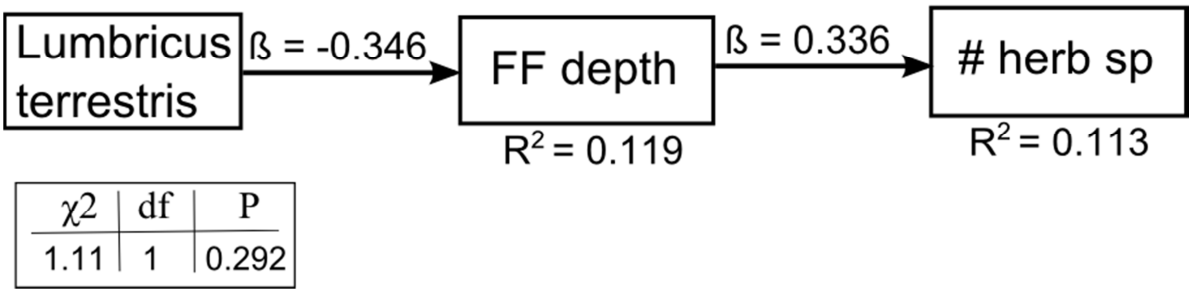


Figure 2: Final path analysis for herbaceous species richness. Arrow sizes represent the strength of the effect (β) and there is only an indirect path between earthworms and species richness. R^2 values are indicated for each endogenous variable. Path coefficients are indicated for each pathway. There was no significant pathway between earthworms and seedlings species richness (not presented).

For the herbaceous community, there was no significant direct effect of earthworms, but we found an indirect effect of *Lumbricus terrestris* abundance on herbaceous species richness via changes in the forest floor (Fig. 2). By significantly reducing the thickness of the forest floor layer, earthworms indirectly reduce herbaceous species richness.

3.5. Discussion

3.5.1. *Earthworms impact on understory composition*

Our sampling of 47 points along a density gradient of exotic earthworms suggests that endogeic and anecic earthworms have an effect on understory vegetation composition even when controlling for confounding factors such as soil properties. In accordance with other studies (Holdsworth et al. 2007; Corio et al. 2009), *Trientalis borealis* cover was negatively correlated with earthworm abundance. Contrary to some earlier findings regarding *Maianthemum canadense* (Hales et al. 2006) and *Dryopteris intermedia* (Corio et al. 2009), we found that these species were also negatively correlated with earthworm abundance. This is consistent with other studies with similar findings to ours (Holdsworth et al. 2007; Nuzzo et al. 2009). Moreover, we found earthworm abundance to be highly correlated with an increased cover of Carex sp. which is consistent with a majority of the literature on the subject (Hales et al. 2006; Powers et al. 2008; Hopfensperger et al. 2011; Drouin et al. 2014). Thus, some species appear to show consistent responses to earthworms across different regions, while others show spatially variable responses, although the latter might be due to differences in methodology (e.g., controlling for potentially confounding variables as we did here).

Several mechanisms might underlie these effects. Our observational study can help to evaluate these potential mechanisms and point to future experimental manipulations that can better test them. The disappearance of the organic layer at high earthworms' densities may explain a decrease in the cover of species that have their roots largely confined to surface organic soil layers, such as *T. borealis* or *D. intermedia* (B. Shipley. U. of Sherbrooke, unpublished database). Furthermore, a possible negative impact of earthworms on root mycorrhizal colonization might decrease the competitive ability of mycorrhizae-dependent plants, which includes most North American herbaceous species (Brundrett et al. 1988). Only endogeic and anecic earthworms had an important effect in our study, as encountered in most other studies

(Corio et al. 2009; Eisenhauer et al. 2009; Nuzzo et al. 2009). Thus, the introduction of epigeic species may be less of a concern for forest plant diversity than the introduction of endogeic and anecic species. This is also consistent with studies showing that *D. octaedra* (an epigeic species) had only small effects on plants (Scheu and Parkinson 1994; Gundale 2002).

3.5.2. *Earthworm impacts on species richness*

We found an indirect effect of *L. terrestris* on the richness of herbaceous species in the forest understory. The reason for this effect seems related to a loss of the organic forest floor. This result might be explained, in part, by the fact that almost all North American herbaceous species are dependent on mycorrhizal associations (Brundrett et al. 1988), which are generally most prevalent in the forest floor layer. Anecic earthworms are a major disturbance agent because of their vertical burrowing up to 2 meters in depth (Bouché 1977). Anecic species bury organic matter deep in the soil and their burrows affect water flow pathways (Edwards and Bohlen 1996; Shipitalo et al. 2004), thereby creating major soil modifications in newly invaded environments. This could explain why we found impacts on soil properties and species richness linked largely to *Lumbricus terrestris*, which is also a potentially important direct consumer of seeds and seedling (Eisenhauer et al. 2010; Griffith et al. 2013). However, since we found no impact of earthworm abundance on tree seedling density, it is possible that seed and seedling predation by *L. terrestris* has yet to have an important impact given the relatively recent invasion of our region (Hale et al. 2005; Nuzzo 2009; Drouin 2014).

3.5.3. *Conclusion*

The aim of this research was to test whether earthworms influence forest understory richness and composition in southern Québec when controlling for bulk soil properties. We found a reduction in herbaceous species richness in the presence of *Lumbricus terrestris* and identified species that appear to be negatively affected by the presence of anecic and endogeic earthworms, *viz*., *T. borealis*, *M. canadensis* and *D. intermedia*. By controlling for confounding factors such as soil pH and dominant vegetation, we are more confident that exotic earthworms – and not correlated environmental factors – are causal agents having an impact on herbaceous communities across eastern North America. This could lead to substantial species turnover and possibly loss of biodiversity, depending on how many species benefit from the decline of earthworm-sensitive plant species.

3.6. Acknowledgements

We thank the following SÉPAQ personnel, Patrick Graillon, Hugues Tennier and Émilie Dorion for their kind support with site access, field lodging, and information about site history. Funding for this project came from Natural Sciences and Engineering Research Council of Canada, Fonds Québécois de la Recherche sur la Nature et Technologies, and SÉPAQ.

3.7. References

Addison, J.A. (2009). Distribution and impacts of invasive earthworms in Canadian forests ecosystems. Biol. Invasions 11: 59-79.

Blanchet, F. G., Legendre, P. & Borcard, D. (2008) Forward selection of explanatory variables. *Ecology* 89: 2623--2632.

Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson, D. (2004). Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 2: 427-435.

Bouché, M.B. (1977). Stratégies lombriciennes. In: Soil Organisms as Components of Ecosystems (eds. U. Lohm & T. Persson), p. 122–132. Ecology Bulletin/NFR, Stockholm

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54: 464-465.

Brundrett, M. C., and Kendrick B. (1988).The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Canadian Journal of Botany* 66.6 : 1153-1173.

Cameron, E. K., Bayne, E. M., Landscape, I., & Group, M. (2007). Human-facilitated invasion of exotic earthworms into northern boreal forests 1-14(4).

Cameron, E. K., Knysh, K. M., Proctor, H. C., & Bayne, E. M. (2013). Influence of two exotic earthworm species with different foraging strategies on abundance and composition of boreal microarthropods. *Soil Biology and Biochemistry*, *57:*334-340.

Corio, K., Wolf, A., Draney, M., & Fewless, G. (2009). Exotic earthworms of great lakes forests: A search for indicator plant species in maple forests. Forest Ecology and Management, 258(7):1059–1066.

Dávalos, A., Nuzzo A., and Blossey B. (2014). Demographic responses of rare forest plants to multiple stressors: the role of deer, invasive species and nutrients. *Journal of Ecology* 102.5: 1222-1233.

Dempsey, M. a., Fisk, M. C., & Fahey, T. J. (2011). Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. Soil Biology and Biochemistry, 43(10), 2135–2141.

Drouin, M., Bradley, R., Lapointe, L., & Whalen, J. (2014). Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. Applied Soil Ecology, 75, 145–149.

Edwards, C. A., and Bohlen. P. J. (1996). *Biology and ecology of earthworms*. Vol. 3. Springer Science & Business Media

Eisenhauer, N., Straube, D., Johnson, E. A., Parkinson, D., & Scheu, S. (2009). Exotic ecosystem engineers change the emergence of plants from the seed bank of a deciduous forest. *Ecosystems*, *12*(6), 1008-1016.

Eisenhauer, N., Butenschoen, O., Radsick, S., & Scheu, S. (2010). Earthworms as seedling predators: importance of seeds and seedlings for earthworm nutrition. *Soil Biology and Biochemistry*, *42*(8), 1245-1252.

Fisk, M. C., Fahey, T. J., Groffman, P. M., & Bohlen, P. J. (2004). Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests.*Ecosystems*, *7*(1): 55-62.

Frelich, L. E., Hale, C. M., Scheu, S., Holdsworth, A. R., Heneghan, L., Bohlen, P. J., & Reich, P. B. (2006). Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions, 8(6), 1235–1245.

Gates GE (1982) Farewell to North American megadriles. Megadrilogica 4:12–77

Griffith, B., Türke, M., Weisser, W. W., & Eisenhauer, N. (2013). Herbivore behavior in the anecic earthworm species Lumbricus terrestris L. *European Journal of Soil Biology*, *55:*62-65.

Gundale, M. J. (2002). Influence of exotic earthworms on the soil organic horizon and the rare fern Botrychium mormo. *Conservation Biology*, *16*(6): 1555-1561.

Hale, C. M., Frelich, L. E., & Reich, P. B. (2006). Changes in hardwood forest understory plant communities in response to European earthworm invasions.*Ecology*, *87*(7): 1637-1649.

Hale, C.M., Host, G.E. (2005) Assessing the impacts of European earthworms invasion in beech-maple hardwood and aspenfir boreal forests of the western Great Lakes region. Great Lakes Network Report GLKN,11: 1–36.

Hendrix P.F., Bohlen P.J. (2002). Exotic earthworm invasions in North America: Ecological and policy implications. BioScience 52:801–11.

Holdsworth, A. R., Frelich, L. E., & Reich, P. B. (2007). Effects of Earthworm Invasion on Plant Species Richness in Northern Hardwood Forests. Conservation Biology, 21(4): 997–1008.

Hopfensperger, K. N., Leighton, G. M., & Fahey, T. J. (2011). Influence of invasive earthworms on above and belowground vegetation in a northern hardwood forest. *The*

American Midland Naturalist, *166*(1):53-62.

Keller, R. P., Cox, A. N., Loon, C. V. A. N., Lodge, D. M., Herborg, L., & Rothlisberger, J. (2006). From Bait Shops to the Forest Floor : Earthworm Use and Disposal by Anglers, 574:321–328.

Kilian, J. V., Klauda, R. J., Widman, S., Kashiwagi, M., Bourquin, R., Weglein, S., & Schuster, J. (2012). An assessment of a bait industry and angler behavior as a vector of invasive species. Biological Invasions, 14(7): 1469–1481.

Lawrence, A. P. & M. P. Bowers, (2002). A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biology & Biochemistry, 34: 549-552.

Lawrence, B., Fisk, M. C., Fahey, T. J., & Suárez, E. R. (2003). Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (Acer saccharum). *New Phytologist*, *157*(1): 145-153.

Legendre, P. & E. D. Gallagher, 2001. Ecologically meaningful transformations for ordination of species data. Oecologia, 129: 271-280.

Nuzzo, V. A., Maerz, J. C., & Blossey, B. (2009). Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. *Conservation Biology*, *23*(4): 966-974.

Powers, M. D., & Nagel, L. M. (2008). Disturbance dynamics influence Carex pensylvanica abundance in a northern hardwood forest 1. *The Journal of the Torrey Botanical Society*, *135*(3): 317-327.

Reynolds J.W. (1977) The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Life Sci Misc Publ, Royal Ont Mus, ON, Canada

Saucier, J.-P. (1994). Le point d'observation ecologique : normes techniques. Ministère des Ressources naturelles du Québec, Québec.

Scheu, S. (2003). Effects of earthworms on plant growth: patterns and perspectives: the 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, *47*(5): 846-856.

Scheu, S., & Parkinson, D. (1994). Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains—laboratory studies. *Applied Soil Ecology*, *1*(2): 113-125.

Shipitalo, M. J., Nuutinen, V., & Butt, K. R. (2004). Interaction of earthworm burrows and cracks in a clayey, subsurface-drained, soil. *Applied Soil Ecology*,*26*(3): 209-217.

Shipley, Bill.(2002). Cause and correlation in biology: a user's guide to path analysis, Cambridge University Press, United Kingdom, 332p.

Tomlin, A. D., and C. A. Fox.(2003). Earthworms and agricultural systems: Status of knowledge and research in Canada. *Canadian journal of soil science,* 83.Special Issue : 265-278.

CHAPITRE 4
DISCUSSION ET CONCLUSION

4.1. *Conclusion*

Dans le cadre de ce projet, nous avons travaillé en partenariat avec la SÉPAQ du Mont-Tremblant afin d'étudier la propagation et les impacts des vers de terre exotiques en milieu forestier. Grâce à l'échantillonnage de 61 lacs, nous avons trouvé qu'en absence d'activité humaine, les vers de terre semblent dans l'incapacité de coloniser rapidement un nouveau milieu. Cela concorde avec l'étude de Marinissen et al. (1992) qui rapportait une migration maximale de 5-10 mètres par année. Dans notre étude, un seul spécimen a été récolté aux lacs éloignés et la consultation de l'historique du parc nous a révélé que le lac où il a été retrouvé était auparavant localisé dans une zone d'exploitation forestière. Ainsi, comme l'étude de Beauséjour 2015 le présentait, il faut considérer les activités humaines du passé pour comprendre pleinement la dispersion actuelle des vers. Notre étude semble montrer qu'il faut une activité humaine permettant de déplacement de vers de terre (pêche, véhicules) dans un rayon de moins de 500 m pour que ceux-ci soient retrouvés à un endroit. Notre hypothèse de base qui supposait une présence des vers aux lacs éloignés surestimait donc leur dispersion. Pour les lacs non pêchés, mais avec une route à proximité, plus du trois quart des vers retrouvés étaient épigéiques. Cela concorde avec l'étude réalisée par Cameron et Bayne (2008) en Alberta qui montrait que les routes étaient principalement un vecteur d'introduction pour *Dendrobaena octaedra.* Enfin, notre étude a permis de supporter notre hypothèse selon laquelle une plus forte densité ainsi qu'une plus forte richesse d'espèces de vers de terre seraient retrouvées autour des lacs pêchés. De plus, l'espèce *Lumbricus terrestris* était uniquement retrouvée autour de ces lacs. Cela concorde avec la littérature qui montre que seule la pêche semble à l'origine des introductions de ce vers anécique (Cameron 2007, Kilian et al. 2012). Lorsque l'on s'éloigne du débarcadère des lacs, on observe une diminution

significative de la densité de vers de terre ce qui suggère fortement que la source d'introduction joue un rôle crucial au niveau de la dispersion. Nous avons aussi attesté que le pH tempère l'abondance des vers. Un sol trop acide agit comme une barrière au développement des populations de vers de terre, comme le suggérait Tiunov et al. (2006) Il s'agit de la première étude à notre connaissance qui vérifie l'effet relatif de la circulation des véhicules par rapport à l'activité de pêche sur la dispersion des vers de terre exotiques dans un lieu donné.

Ces informations seront utiles dans le cadre de la gestion des milieux protégés. Par exemple en restreignant la circulation des véhicules à certaines routes ou en limitant la pêche à certains secteurs du parc. Ainsi on pourrait éviter la progression des fronts d'invasions des vers de terre exotiques. En ce sens, il serait intéressant que les gestionnaires du parc du Mont-Tremblant fassent un suivi à long terme de l'état d'invasion des vers de terre. Il serait également utile d'analyser la dispersion des vers en fonction de l'année d'ouverture d'un lac à la pêche ou de la construction d'une route. De plus, il serait intéressant d'évaluer la dispersion spatiale des vers de terre à de plus fines échelles afin de détecter des changements annuels des communautés végétales le long des fronts d'invasion.

Il était important pour nos partenaires de la SÉPAQ d'obtenir une réponse claire quant aux impacts des vers de terre exotique sur le territoire qu'ils ont pour mandat de protéger. Nos hypothèses de départ étaient que la présence de vers de terre en milieu forestier serait associée à une augmentation de la production d'oxyde nitreux, une diminution de la richesse et de la densité de la végétation de sous-bois et par conséquent à un changement dans la composition des espèces de sous-bois. Bien que nos hypothèses soient attestées par nos données, , elles ont soulevées de nouvelles questions et réponses. Par exemple, seuls les vers anéciques (*Lumbricus terretris)* étaient associés à une augmentation de la production d'oxyde nitreux et à une diminution de la richesse des herbacées. Les vers anéciques semblent provoquer ces changements en modifiant le ratio C/N et la profondeur de l'horizon F. Pareillement, notre

étude a montré que la combinaison des vers endogés et anéciques avait un plus grand impact sur la composition de la strate herbacée (e.g. diminution de *M. canadensis*, *T. borealis* et *D. intermedia*; augmentation des *Carex* sp.). Il se pourrait que ces effets s'amplifient davantage puisque les invasions de vers de terres au Parc du Mont-Tremblant semblent relativement récentes (voir résultat premier volet).

La principale faiblesse de notre échantillonnage fut de ne pas avoir réussi à inclure dans l'échantillonnage une zone sans le moindre vers de terre à des fins de comparaison. Avec plus de temps, il aurait fallu trouver le front d'invasion des vers de terre pour échantillonner de part et d'autre de celui-ci afin de pouvoir comparer les données avec et sans vers de terre plutôt que sur un gradient de présence de vers de terre n'incluant pas l'absence de vers de terre.

4.2. *Perspectives du projet*

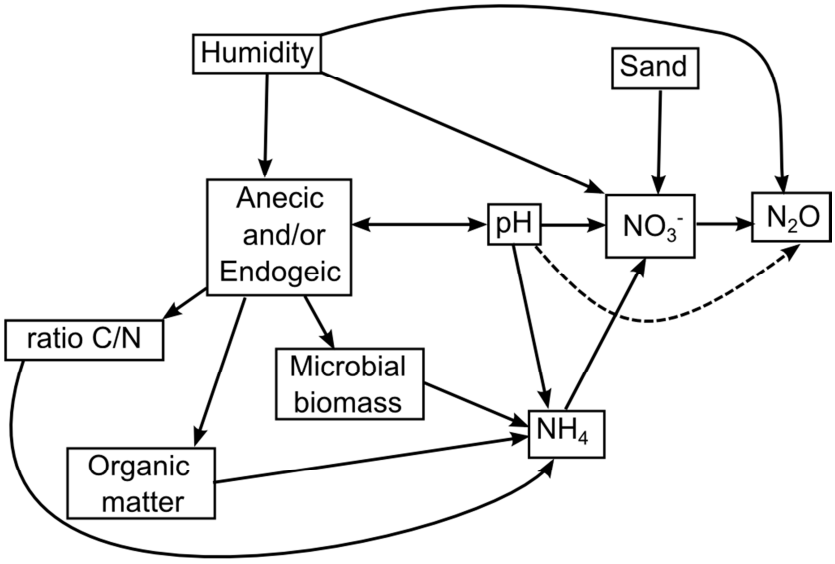
En établissant que les vers exotiques ont des impacts négatifs dans le parc du Mont-Tremblant, notre étude illustre la nécessité de la mise en place de mesures pour contrôler leur propagation. Selon l'état actuel des choses dans le parc, *Lumbricus terrestris* est l'espèce exotique de vers de terre à surveiller étant donné ses impacts au niveau de la richesse en herbacées et de la production d'oxyde nitreux. En montrant que les vers anéciques ne sont introduits que près des zones fortement pêchés, notre étude offre une information importante aux gestionnaires de ce milieu protégé. Mon projet est le commencement d'une campagne de sensibilisation au Québec. Nous travaillons présentement avec les responsables de la conservation du parc afin de créer des dépliants qui seront distribués aux pêcheurs afin de les sensibiliser pour qu'ils ne relâchent plus leurs appâts vivants.

Les mécanismes derrières les changements provoqués par les vers de terre au niveau de la végétation demeurent encore mal compris. Il serait intéressant pour un projet futur d'instaurer des parcelles semi-permanentes afin de pouvoir faire un suivi des caractéristiques dynamiques du sol et de la végétation pour mieux cerner pourquoi certaines espèces semblent affectées par la présence de vers et d'autres non. Il serait également intéressant dans un futur proche de poursuivre ce genre d'étude afin d'étudier la dispersion des vers de terre et leurs impacts dans d'autres milieux protégés. Ainsi il sera possible de vérifier si les conclusions de notre étude sont également applicables aux autres milieux forestiers du Québec. Cela permettra au besoin d'établir un plan de gestion de la propagation des vers de terre exotiques pour éviter que nos écosystèmes forestiers déjà affaiblis par les changements climatiques ne subissent des perturbations supplémentaires (Aber et al. 2001). Selon la loi sur les parcs : « Un parc national a pour objectif prioritaire d'assurer la conservation et la protection permanente de territoires représentatifs des régions naturelles du Québec…», il sera donc du devoir de la SÉPAQ d'assurer la protection de son patrimoine face aux invasions des vers de terre exotiques.

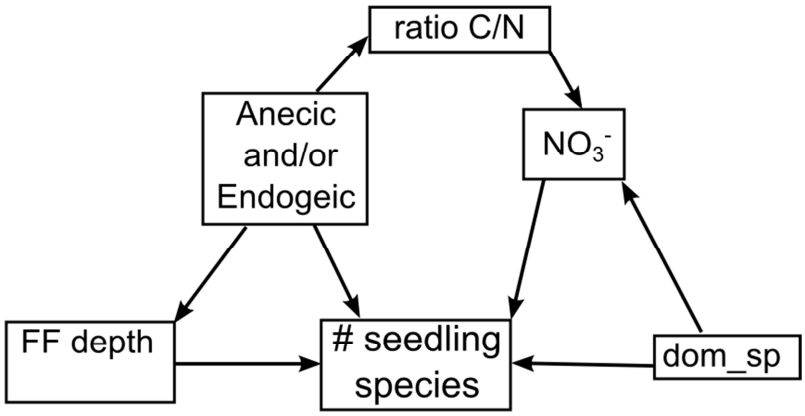
ANNEXES

Appendix A: Complete initial DAG built according to literature

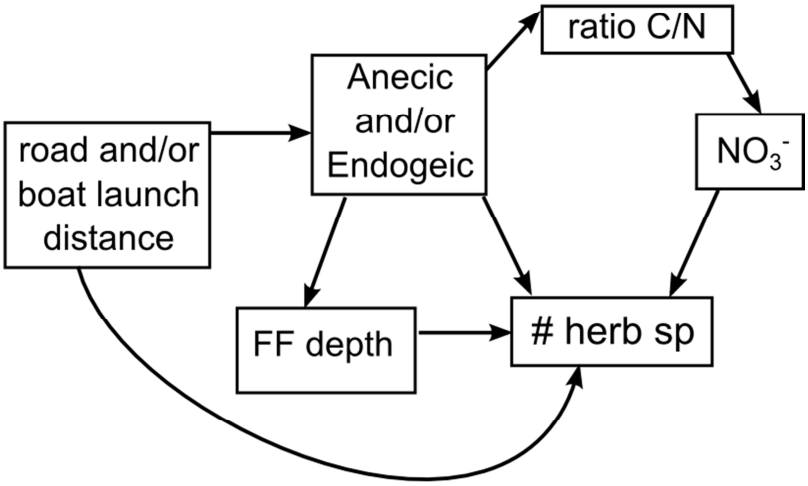
Denitrification initial DAG



Seedling species richness initial DAG



Herbaceous species richness initial DAG



BIBLIOGRAPHIE

Aber J.D., Neilson R.P., McNulty S., Lenihan J.M., Bachelet D., Drapek R.J. (2001). Forest processes and global environmental change; predicting the effects of individual and multiple stressors. BioScience 51:735–51.

Addison, J.A. (2009). Distribution and impacts of invasive earthworms in Canadian forests ecosystems. Biol. Invasions 11: 59-79.

Alban, D.H. & Berry, E.C. (1994) Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. *Applied Soil Ecology*, 1, 243–249.

Baker, G.H., Brown, G., Butt, K.R., Curry, J.P. & Scullion, J. (2006). Introduced earthworms in agricultural and reclaimed land: their ecology and influences on soil properties, plant production and other soil biota. Biological Invasions, 8:1301–1316.

Beauséjour, R., Handa, I. T., Lechowicz, M. J., Gilbert, B., & Vellend, M. (2015). Historical anthropogenic disturbances influence patterns of non-native earthworm and plant invasions in a temperate primary forest. *Biological Invasions*, *17*(4), 1267-1281.

Bohlen, P. J., Pelletier, D. M., Groffman, P. M., Fahey, T. J., & Fisk, M. C. (2004). Influence of Earthworm Invasion on Redistribution and Retention of Soil Carbon and Nitrogen in Northern Temperate Forests. *Ecosystems*, *7*(1), 13–27.

Bohlen, P.J., Scheu, S., Hale, C.M., McLean, M.A., Migge, S., Groffman, P.M., Parkinson, D. (2004). Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 2: 427-435.

Bouché, M.B. (1977). Stratégies lombriciennes. In: Soil Organisms as Components of Ecosystems (eds. U. Lohm & T. Persson), p. 122–132. Ecology Bulletin/NFR, Stockholm

Brundrett, Mark C., and Bryce Kendrick. (1988).The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Canadian Journal of Botany* 66.6 : 1153-1173.

Burtelow AE, Bohlen PJ, Groffman PM (1998) Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Appl Soil Ecol 9:197–202

Cameron, E. K., Bayne, E. M., & Coltman, D. W. (2008). Genetic structure of invasive earthworms Dendrobaena octaedra in the boreal forest of Alberta: insights into introduction mechanisms. *Molecular ecology*, *17*(5), 1189–97.

Cameron, E. K., Bayne, E. M., Landscape, I., & Group, M. (2007). Human-facilitated invasion of exotic earthworms into northern boreal forests 1, *14*(4).

Clapp, J., Helleiner, E., Hester, A., Homer-Dixon, T., Rowlands, H.I., Swanston, L., Thstlethwaite, J., VanNijnatten, L.D. and Whalley, J. (2009). Environmental Sustainability and the Financial Crisis: Linkages and Policy Recommendations. Centre for International Governance Innovation (CIGI), Waterloo, Ontario, Canada, 38p.

Corio, K., Wolf, A., Draney, M., & Fewless, G. (2009). Exotic earthworms of great lakes forests: A search for indicator plant species in maple forests. *Forest Ecology and Management*, *258*(7), 1059–1066.

DeFauw, S. L., Vogt, J. T., & Boykin, D. L. (2008). Imported fire ant (Hymenoptera: Formicidae) bioturbation and its influences on soils and turfgrass in a sod production agroecosystem. *Journal of entomological science*,*43*(1), 121.

Dempsey, M. a., Fisk, M. C., & Fahey, T. J. (2011). Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. *Soil Biology and Biochemistry*, *43*(10), 2135–2141.

Drouin, M., Bradley, R., Lapointe, L., & Whalen, J. (2014). Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. Applied Soil Ecology, 75, 145–149.

Edwards CA (Ed). 2004. Earthworm ecology, 2nd edn. Boca Raton: CRC Press.

Edwards, Clive A., and Patrick J. Bohlen. (1996). *Biology and ecology of earthworms*. Vol. 3. Springer Science & Business Media

Ehrenfeld J.G., Kourtev P., Huang W.Z. (2001). Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecol Appl 11:1287–300
.
Eisenhauer, N., Straube, D., Johnson, E. A., Parkinson, D., & Scheu, S. (2009). Exotic ecosystem engineers change the emergence of plants from the seed bank of a deciduous forest. *Ecosystems*, *12*(6), 1008-1016.

Ewing, H. a., Tuininga, A. R., Groffman, P. M., Weathers, K. C., Fahey, T. J., Fisk, M. C., Bohlen, P. J., et al. (2014). Earthworms Reduce Biotic 15-Nitrogen Retention in Northern Hardwood Forests. *Ecosystems*, *18*(2), 328–342.

Fisk, M. C., Fahey, T. J., Groffman, P. M., & Bohlen, P. J. (2004). Earthworm invasion,

fine-root distributions, and soil respiration in north temperate forests.*Ecosystems*, *7*(1): 55-62.

Frelich, L. E., Hale, C. M., Scheu, S., Holdsworth, A. R., Heneghan, L., Bohlen, P. J., & Reich, P. B. (2006). Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions*, *8*(6), 1235–1245.

Gates G.E. (1982) Farewell to North American megadriles. Megadrilogica 4:12–77

Giannopoulos, G., Pulleman, M. M., & Van Groenigen, J. W. (2010). Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N2O dynamics in agricultural soil. *Soil Biology and Biochemistry*, *42*(4), 618–625

Groffman, P.M., Bohlen, P.J., Fisk, M.C., Fahey, T.J., (2004). Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7, 45–54.

Hale, C. M., Frelich, L. E., & Reich, P. B. (2006). Changes in hardwood forest understory plant communities in response to European earthworm invasions.*Ecology*, *87*(7): 1637-1649.

Hale C.M., Host G.E. (2005) Assessing the impacts of European earthworms invasion in beech-maple hardwood and aspenfir boreal forests of the western Great Lakes region. Great Lakes Network Report GLKN,11: 1–36.

Hendrix P.F., Bohlen P.J. (2002). Exotic earthworm invasions in North America: Ecological and policy implications. BioScience 52:801–11

Holdsworth, A. R., Frelich, L. E., & Reich, P. B. (2007). Effects of Earthworm Invasion on Plant Species Richness in Northern Hardwood Forests. *Conservation Biology*, *21*(4), 997–1008.

Karsten, G. R., & Drake, H. L. (1997). Denitrifying Bacteria in the Earthworm Gastrointestinal Tract and In Vivo Emission of Nitrous Oxide (N (inf2) O) by Earthworms. *Applied and Environmental Microbiology*, *63*(5), 1878-1882.

Kilian, J. V., Klauda, R. J., Widman, S., Kashiwagi, M., Bourquin, R., Weglein, S., & Schuster, J. (2012). An assessment of a bait industry and angler behavior as a vector of invasive species. *Biological Invasions*, *14*(7), 1469–1481.

Kuiper, I., de Deyn, G. B., Thakur, M. P., & van Groenigen, J. W. (2013). Soil invertebrate fauna affect N2 O emissions from soil. *Global change biology*, *19*(9), 2814–25.

Lavelle, P., Brussaard, L., & Hendrix, P. (1999). *Earthworm management in tropical*

agroecosystems. CABI publishing.

Lavelle, P. (1988). Earthworm activities and the soil system. *Biology and fertility of soils*, *6*(3), 237-251.

Lawrence, B., Fisk, M. C., Fahey, T. J., & Suárez, E. R. (2003). Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (Acer saccharum). *New Phytologist*, *157*(1): 145-153.

Lee, K. E. (1985). *Earthworms: their ecology and relationships with soils and land use*. Academic Press Inc..

Marhan, S., Auber, J., & Poll, C. (2015). Additive effects of earthworms, nitrogen-rich litter and elevated soil temperature on N2O emission and nitrate leaching from an arable soil. *Applied Soil Ecology*, *86*(3), 55–61.

Marinissen JCY, van den Bosch F (1992) Colonization of new habitats by earthworms. Oecologia 91:371–376

McKey-Fender D., Fender W.M., Marshall V.G. (1994). North American earthworms native to Vancouver Island and the Olympic Peninsula. Can J Zool 72:1325–1339

McLean, M. a., Migge-Kleian, S., & Parkinson, D. (2006). Earthworm invasions of ecosystems devoid of earthworms: effects on soil microbes. *Biological Invasions*, *8*(6), 1257–1273.

Moore J-D, Reynolds JW (2003) Premières mentions de vers de terre (Oligochaeta: Lumbricidae) dans deux écosystèmes forestiers du bouclier canadien, Quebec, Canada. Megadrilogica 9:53–59

Perez-Moreno, J., & Read, D. J. (2000). Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytologist*, *145*(2), 301-309.

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological economics*, *52*(3), 273-288.

Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., ... & Midgley, P. M. (2014). Climate change 2013: The physical science basis. T. Stocker (Ed.). Cambridge, UK, and New York: Cambridge University Press, 1535p.

Reynolds J.W. (1977) The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Life Sci Misc Publ, Royal Ont Mus, ON, Canada

Satchell, J. E. (1983). Earthworm microbiology. In Earthworm Ecology (pp. 351-364). Springer Netherlands.
Scheu, S., & Parkinson, D. (1994). Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains—laboratory studies. *Applied Soil Ecology*, *1*(2): 113-125.

Scheu, S. (2003). Effects of earthworms on plant growth: patterns and perspectives: the 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, *47*(5): 846-856.

Migge S. 2001. The effect of earthworm invasion on nutrient turnover, microorganisms and microarthropods in Canadian aspen forest soil (PhD dissertation). Darmstadt, Germany: Technische Universität Darmstadt.

Suárez, E. R., Fahey, T. J., Yavitt, J. B., Groffman, P. M., & Bohlen, P. J. (2006). Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. *Ecological Applications*, *16*(1), 154-165.

Tiunov, A. V., C. M. Hale, A. R. Holdsworth & T. S. Vsevolodova-Perel, 2006. Invasion patterns of Lumbricidae into the previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. Biological Invasions, 8: 1223-1234.

Van Driesche J. et Van Driesche R. (2000).*Nature Out of Place: Biological Invasions inthe Global Age, Washington (DC), Island Press, 352p.*

Vitousek PM. (1990). Biological invasions and ecosystem processes: toward an integration of population biology and ecosystem studies. *Oikos* 57: 7–13.